THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR Sue Jane Eberlein

TITLE OF THESIS A Genetic Analysis of the *engrailed* Region of *Drosophila melanogaster*

DEGREE FOR WHICH THESIS WAS PRESENTED Doctor of Philosophy

YEAR THIS DEGREE GRANTED Fall 1983

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

THE UNIVERSITY OF ALBERTA

A Genetic Analysis of the *engrailed* Region of *Drosophila melanogaster*

by

Sue Jane Eberlein

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Doctor of Philosophy

Department of Genetics

EDMONTON, ALBERTA

FALL 1983

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled A Genetic Analysis of the *engrailed* Region of *Drosophila melanogaster* submitted by Sue Jane Eberlein in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

To my parents, without whom it never would have been possible.

ABSTRACT

The *engrailed* gene of *Drosophila melanogaster* is believed to be involved in control of determination and differentiation of posterior compartments. *en*^{1}/*en*^{1} causes a partial transformation of the posterior compartment of wing and first leg to mirror image anterior, which prompted the hypothesis that *engrailed* is a "selector gene" required for the posterior pathway decision. The incomplete transformation was thought due to residual *en*+ activity in *en*^{1}. I have tested this hypothesis by analysing the effects of a deletion of the locus (*en28*), and another deletion in the region (*en30*) which survives over lethal *engrailed* alleles. Both deletions are homozygous lethal and cell lethal. *en28*/*en*^{1}, *en30*/*en*^{1}, and *en28*/*en30* all survive to adult stage, but no genotype causes a stronger posterior to anterior transformation than *en*^{1}/*en*^{1}, suggesting that this effect is allele specific and that *en*^{1} is not a simple hypomorph. New abnormalities included: 1) transformation of posterior wing blade to haltere, an effect dependent on the *bx*+ (but not *pbx*+) pseudoallele of the Bithorax-complex. 2) abnormal bristle pattern, tarsal fusion and degenerate posterior claws of all legs. Selection for strong expression of posterior to anterior transformation may result in increased expression of the new abnormalities, and both types of abnormality can coexist in the same structure. Abnormalities resembling cell death defects occur in genitalia, and in the heads of a single strain of *en*^{1}/*en28*. Alterations in the haltere are also observed in some backgrounds; thus *engrailed* may be required in all imaginal discs. Although adult abnormalities are posterior compartment specific, many are not expected of a "selector gene", and alternative hypotheses for the function of *engrailed* are considered.

ACKNOWLEDGEMENTS

I wish to extend my deepest thanks to my supervisor, Dr. Michael A. Russell, for his guidance, encouragement, and great patience throughout the course of this work. For their friendship and for many stimulating discussions, I thank Pliny Hayes, Jim Kennison, Stanley Tiong, Emil Steiner, John Williams, and Asma Zaidi. I thank my husband, Loring Craymer, for his encouragment and support during the writing of this thesis.

Table of Contents

Chapter Page

List of Tables

List of Figures

INTRODUCTION

Like other higher organisms, Drosophila melanogaster develops from a single fertilized egg to a multi-cellular, highly differentiated adult. A crucial question in developmental biology is how genes direct cells into the correct developmental pathways resulting in the formation of the adult structures.

During the first three hours of development of the Drosophila egg at 25°, nine nearly synchronous cycles of nuclear division occur without intervening formation of cells membranes or cytoplasmic division (Sonnenblick, 1950; Zalokar and Erk, 1976). Nuclei at the 256-nucleus stage are still totipotent; if removed from the egg and transplanted into another cleaving egg, they can participate in the formation of any of the adult structures including the germ line (Zalokar, 1971; Okada et al., 1974).

By the end of the first nine cycles of nuclear division, most of the nuclei have migrated to the periplasm of the egg where they undergo four further divisions (Zalokar and Erk, 1976; Foe and Alberts, 1983). This period is known as the syncitial blastoderm, generally occurring between two and three hours after egg laying. During the first half of the interphase following the thirteenth nuclear division, cell membranes are formed simultaneously between all the nuclei lining the egg periphery in a single layer (Foe and Alberts, 1983). Using an electron microscope to count nuclei, Turner and Mahowald (1976) estimated that 6500 nuclei were incorporated into cells. The resulting cellular blastoderm immediately begins gastrulation (Foe and Alberts, 1983).

If nuclei from this stage are transplanted without accompanying cytoplasm, they can participate in the development of the germ line in a fertilized egg (Zalokar, 1973). However, entire cells are not totipotent. Cells of the anterior blastoderm will form only anterior adult structures following dissociation and culture; posterior blastoderm cells form only posterior adult structures (Chan and Gehring,

1971). When transplanted, blastoderm cells give rise to imaginal structures according to position of origin rather than their new position (reviewed in Illmensee, 1978; Simcox and Sang, 1983). Thus some characteristic of the cortical cytoplasm is responsible for directing the behavior of cells.

Further evidence of the role of the cytoplasm in determining developmental fates of cells is presented when posterior cytoplasm (without nuclei) is transplanted to the anterior pole of a cleaving egg. This results in production of normal germ cells at the anterior pole (Illmensee and Mahowald, 1974). The existence of maternal effect mutations which alter embryonic pattern suggests that the cytoplasmic characteristics are provided by the maternal genome (Nusslein-Volhard, 1979; Rice and Garen, 1975). For example, mothers mutant for bicaudal (Nusslein-Volhard, 1979) or dicephalic (Lohs-Schardin, 1982) produce embryos with abnormal pattern arrangement in the anterior-posterior axis. The maternal effect mutant dorsal causes pattern alteration in the dorsal-ventral axis (Nusslein-Volhard et al., 1980). It is postulated that these mutant maternal genomes are incapable of producing or distributing morphogens which establish the initial spatial organisation of the egg. Possibly gradients of such morphogens provide cells with information regarding their position in the developing system (Nusslein-Volhard, 1979; Wolpert, 1969, 1971; Wolpert and Lewis, 1975; for review see Russell and Hayes, 1980). In addition to the correct cytoplasmic characteristics, proper development requires communication between regions of the developing egg. Ligation of the egg to prevent diffusion between anterior and posterior regions results in embryos which lack the central segments (Schubiger et al., 1977).

Incorporation of the blastoderm nuclei into cells marks the beginning of a change in the nature of development. While pre-cellular blastoderm nuclei are totipotent, cells show a restriction in developmental potential. This kind of restriction

is referred to as "determination". Hadorn (1965, p 148) defined determination as "...a process which initiates a specific pathway of development by singling it out from among various possibilities for which a cellular system is competent". An important aspect of determination is that the determined state is heritable.

The cell transplantation experiments of Simcox and Sang (1983) suggest that blastoderm cells are determined to within a segment. Further evidence for blastoderm cell determination is presented in the reaction of the egg to local damage. Destruction of cells of the cellular blastoderm (Bownes and Sang, 1974a,b; Lohs-Schardin et al., 1979a,b) results in larval or adult abnormalities corresponding to the position of damage. Such damage may eliminate all adult derivatives of a segment. Thus the surrounding cells cannot regenerate all the structures which would have been produced by the damaged cells. This technique has been used to construct a "fate map" of the blastoderm cells, indicating which blastoderm regions will produce specific embryonic, larval and adult structures (Lohs-Schardin et al., 1979a,b; Underwood et al., 1980).

A problem arises in studying determination in that the determinative event itself sometimes takes place long before its effect is expressed in differentiation. Although a cell at the blastoderm stage may be determined to form only anterior imaginal structures, these structures will not actually be observed until metamorphosis has occurred many hours later. Drosophila development presents many examples of clear determinative events occurring long before terminal differentiation. Throughout larval development, the cells which will eventually form the adult structures are sequestered in imaginal discs and abdominal histoblasts (Auerbach, 1936; Bodenstein, 1950). These cells do not differentiate until metamorphosis (for reviews see Nothiger, 1972; Russell, 1982).

The imaginal discs of a third instar larva are clearly determined to form

specific structures. The discs can be recognized by morphology and position in the larva (Bodenstein, 1950). If an entire disc is transplanted to another larva and allowed to metamorphose, it will form the same inventory of structures that it would have formed in situ (for review see Gehring and Nothiger, 1973). Adult tissues normally produced by other discs are not generally found. This indicates that the determined state of the third instar disc cells cannot be changed by altering the disc's environment. If the disc is fragmented or damaged, parts of the disc will form specific structures. Using these techniques it has been possible to construct detailed fate maps of the different disc regions (Hadorn and Buck, 1962; Schubiger, 1968; for review see Gehring and Nothiger,1973).

There is some evidence that determination of imaginal structures is not entirely mosaic even at the third larval instar, since removal of some cells can still be compensated for by regulation of other cells, provided extra cell divisions are allowed. If a disc fragment is allowed to grow in culture before metamorphosing, it can regenerate elements which would not normally be formed by that fragment (Schubiger, 1971; Bryant,1975; Strub, 1977). The inventory of structures formed still does not normally extend beyond the single disc, although when excessive growth is allowed, groups of cells may be redetermined to form another structure. This has been termed "transdetermination" (Hadorn, 1965, 1978).

These methods of studying determination encounter problems in that disc damage and transplantation may disrupt development, creating situations that do not parallel the normal state. They are also limited to discs of the second or third larval instar. By this time the discs are clearly separate entities in the larva (Auerbach, 1936); the events that led to the determination of the disc cells must have occurred much earlier.

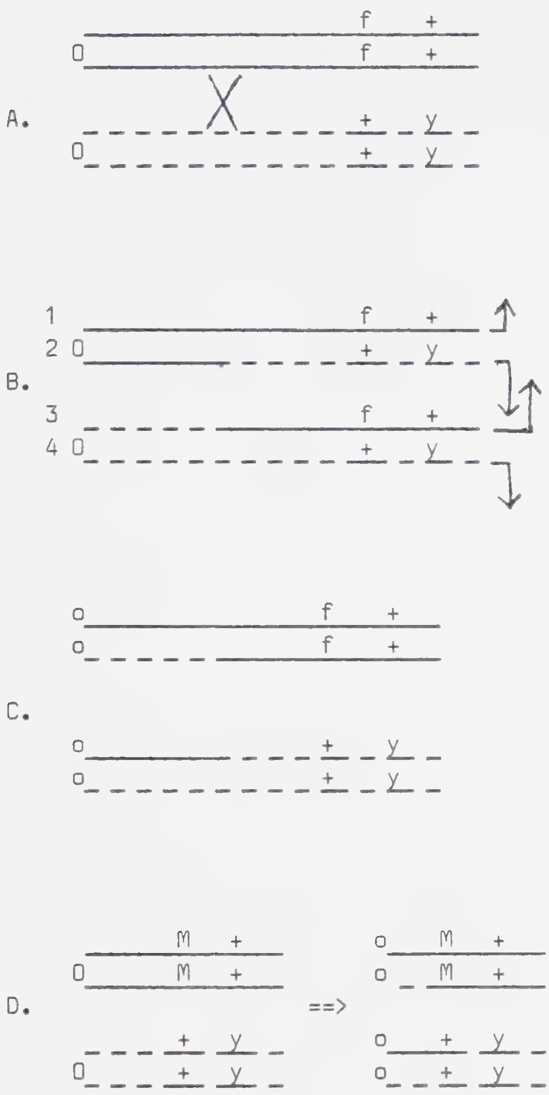
Several techniques have been developed for studying early determinative events.

These techniques involve the marking of single cells early in development such that their fates can be followed through terminal differentiation. The technique which is most significant to the present study is clonal analysis. This analysis is based on the observation that recombination between two homologues at the four strand stage of mitosis can result in daughter cells homozygous for the genetic markers distal to the recombination event (Figure 1). Since all the progeny of such cells will also be homozygous for the markers, the result is an identifiable clone of marked tissue on the adult cuticle. If different recessive markers are present on both homologues, twin spots can be identified on the adult.

Somatic recombination was first thoroughly investigated by Stern (1936), who found that some _Minute_ mutations (a class of mutations named for producing small bristles) could increase the level of recombination over the very low spontaneous frequency occurring in wild type flies. Later it was discovered that irradiation of larvae at various stages of development could induce somatic recombination (Becker, 1957). This provided a technique for determining the time at which the recombination event occurred, and made it possible to infer the developmental potential of a single cell at a given stage. By superimposing the outlines of clones found in several flies, it could be seen that even when induced very late in development, and comprising only a few cells, clones overlapped. This indicated that there was not an absolutely fixed cell lineage in a structure. Inclusion of two pattern elements in a single clone could show that the founding cell of the clone had not been determined to produce only one of the elements. However, the failure of a single clone to encompass any two pattern elements could not be taken to indicate that the progenitor cell was so determined. The limiting factor could be small clone size.

The problem of size limitation was partly overcome by an additional technique, the production of _Minute_$^+$ (_M_$^+$) clones in a _Minute_ background (Figure 1D; Morata and

Figure 1. Somatic recombination for clonal analysis.



A. Four-strand stage of mitosis: each homologue carries two chromatids, and must pair for recombination to occur. Recombination pictured proximal to both *forked* (*f*) and *yellow* (*y*).

B. One of two possible segregation patterns of chromatids at anaphase: 1,3 disjoin from 2,4 leading to the two daughters shown in C. If 1,4 disjoin from 2,3 the result is that both daughters cells are *y* +/+ *f*.

C. One daughter is homozygous for *f*, the other for *y*. Each will grow to form a clone of marked tissue, resulting in a twin spot.

D. *M* homozygote dies

M^+ homozygote in *M*/+ background grows faster than surrounding cells to produce very large clone.

Ripoll, 1975). Minute cells divide less frequently than wild type, and the $\underline{M}^+$ cells of the clone will overgrow the surrounding cells. This technique makes it possible to estimate more closely the full developmental potential of a single cell at a given stage. Using this method, Wieschaus and Gehring (1976) showed that clones induced at blastoderm would not transgress the boundaries between segments in the longitudinal axis of the animal, although very large clones might cross between the dorsal and ventral derivatives of a single segment. Thus the blastoderm cells which would form a specific adult segment had been determined for that segment before the onset of the cell divisions which would produce the imaginal discs.

In addition to respecting the boundaries between segments, post-blastoderm clones also defined a dividing line within some of the thoracic segments (Garcia-Bellido, Ripoll and Morata, 1973, 1976; Wieschaus and Gehring, 1976; Steiner, 1976). This line separated the anterior from the posterior portions of a disc's derivatives; the same dividing line was clearly defined by many clones. Garcia-Bellido, Ripoll and Morata (1973) termed these subdivisions "compartments". In the wing, a large clone could run along the anterior-posterior compartment boundary for hundreds of cells without crossing the boundary into the other compartment (Garcia-Bellido, Ripoll and Morata, 1973, 1976). Brower et al. (1981) also visualized this boundary in the third instar wing disc, by inducing clones of cells with differential staining properties. The anterior-posterior boundary in the adult legs, although clearly defined by most clones, was not always as strictly respected as in the wing (Steiner, 1976). An anterior-posterior boundary was also formed at blastoderm in the proboscis (Struhl, 1977, 1981). It is noteworthy that even very large clones were never found to fill completely either the anterior or posterior compartment. This indicates that a group of cells rather than one cell is involved in the decision to become a compartment. Crick and Lawrence (1975) have termed such groups of progenitor cells "poly clones".

By inducing clones at later stages in development it was shown that compartment subdivisions occur, dividing the wing disc derivatives into dorsal/ventral and wing/notum compartments (Figure 2A,B; Garcia-Bellido, Ripoll and Morata, 1973, 1976). Clones induced later than 72 hours after egg laying (AEL) in Minute animals also indicate boundaries in the eye-antenna disc derivatives (Morata and Lawrence, 1978, 1979) and possibly the genitalia (Wieschaus and Nothiger, 1982; Epper and Kornberg, personal communication).

It has been speculated that these compartments are in some way significant in the control of development. For example, each compartment might be characterized by a unique pattern of gene activity. Garcia-Bellido (1975) developed this idea when he introduced the "selector gene" model. According to this model, a selector gene is to be thought of as a regulatory locus which controls batteries of other structural or "cytodifferentiation" genes. He proposed that each of a series of compartmentalization events might occur by the permanent activation of a selector gene in one compartment, leaving the gene inactive in the other compartment. The application of this model to the wing disc (as envisioned by Lawrence and Morata, 1976) is illustrated in Figure 2D. The formation of the anterior and posterior compartments results from turning gene X on only in those cells destined to form the posterior compartment (Figure 2D); gene Y is turned on only in the cells destined to form ventral compartment and so on.

Kauffman (1977) proposed a biochemical basis for compartment formation. If a biochemical system were undergoing reaction and diffusion in the growing disc, a series of concentration patterns would result. Kauffman postulated that a threshold concentration of a specific chemical would turn on a bistable switch. This model was originally applied to the segmentation of the embryo (Kauffman, 1973, 1975), and used to explain the observed order of transdetermination in cultured discs (Hadorn, 1965,

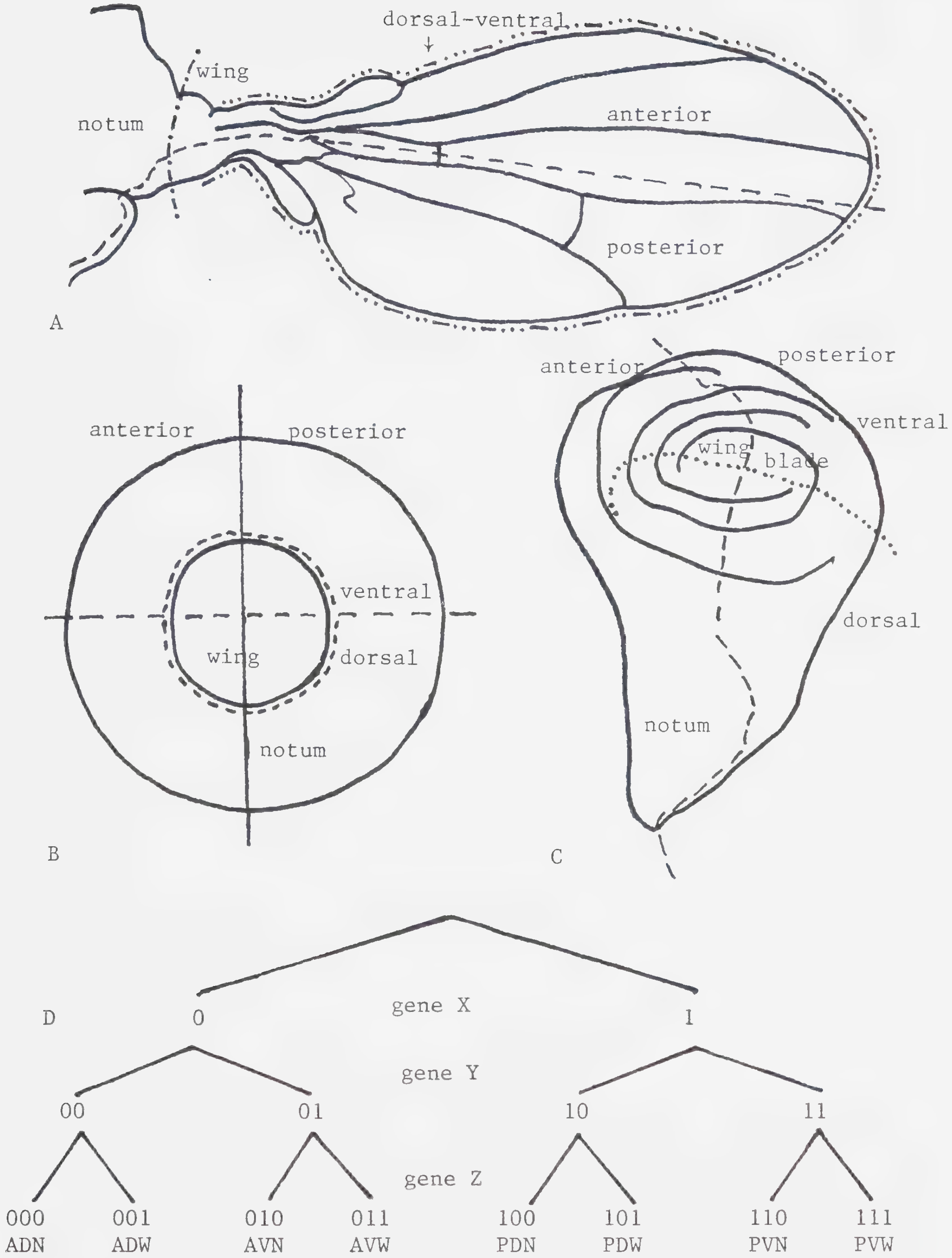
Figure 2. Compartment formation in the wing disc.

A. Location of compartment boundaries on the adult derivatives of the dorsal mesothoracic disc. Dashed line shows anterior-posterior compartment boundary, formed at blastoderm. Dotted and dashed line shows wing notum boundary; triple dotted and dashed line shows boundary between dorsal and ventral surfaces of wing blade. Both restrictions form around 96-120 h AEL in Minute background. (After Garcia-Bellido et al., 1976; Bryant, 1975.)

B. Schematic diagram of how restrictions in the wing disc divide it into anterior and posterior (solid line), dorsal and ventral (dashed line), and wing and notum (solid and dashed) compartments. (After Garcia-Bellido et al., 1976.)

C. Diagram of the mature wing disc. Compartment boundaries are as determined by clonal analysis of the disc using the cell marker succinate dehydrogenase (Lawrence, 1981b). Dashed line shows anterior-posterior compartment (Brower et al. 1981). Dotted line shows dorsal-ventral compartment boundary (Brower et al. 1982). Wing and notum precursors are as determined by fate mapping (Bryant, 1975).

D. Control of compartment identity by a series of selector genes. Each of three genes can be on (1) or off (0), allowing for eight unique combinations. (After Lawrence and Morata, 1976.)
gene X: 0 = anterior (A) 1 = posterior (P)
gene Y: 0 = dorsal (D) 1 = ventral (V)
gene Z: 0 = notum (N) 1 = wing (W)


dorsal-ventral
wing
notum
anterior
posterior
A
anterior
posterior
ventral
wing
dorsal
notum
B
anterior
posterior
ventral
wing
blade
dorsal
notum
C
D
gene X
0
1
gene Y
00
01
10
11
gene Z
000
ADN
001
ADW
010
AVN
011
AVW
100
PDN
101
PDW
110
PVN
111
PVW

1978). When applying the model to discs, Kauffman (1977) found that chemical patterns in a growing circle could actually produce thresholds which correspond to the compartment boundaries observed in the wing disc (Garcia-Bellido, Ripoll and Morata, 1976). Kauffman's bistable circuits correspond to the selector genes of Garcia-Bellido's model.

According to the model of Garcia-Bellido, a selector gene would serve several functions:

1) If the state of the gene were heritable, it would act as a memory of the early decision, possibly by some kind of feedback mechanism. During subsequent cell divisions, the progeny of the posterior cells (Figure 2D) would also have gene X turned on, and thus would be marked as posterior.

2) The selector gene would direct the cells down the proper developmental pathway, by activating a specific subset of genes. In the case of the anterior-posterior compartment decision, cells which had selector gene X turned off would develop and differentiate as anterior structures; with gene X on, the anterior pathway would be repressed and the posterior pathway followed. If a series of compartmentalization events sequentially affected a group of cells (as in the wing disc), each single compartment would be determined by the combination of selector genes turned on in the cells. Proper differentiation at metamorphosis would depend on the specific combination of selector genes activated.

As a subset of this function, the selector gene might be responsible for allowing cells to recognize and preferentially associate with other cells of the same compartment. There is evidence from several systems that different cell types, when mixed and cultured, will sort out from each other into groups of like cells (see Steinberg, 1964). Such cell behavior does not require any special mechanism of directed cell migration; greater average affinity between like cells than between

unlike is sufficient to explain the observations (Steinberg, 1964). This could be attributed to a simple difference in cell surface characteristics directed by a selector gene. There is some evidence that Drosophila cells from different discs or from different compartments of the same disc will sort out from each other after dissociation and mixing (Garcia-Bellido, 1966). However, the apparent sorting behavior could also be attributed to incomplete dissociation, or to division of a few cells of the same type producing a cluster of like cells (Poodry, Bryant and Schneiderman, 1971). If the cell sorting is real, it could explain the formation of straight compartment boundaries; a straight boundary would occur if cells from the two different compartments were minimizing the area of interface between them (Crick and Lawrence, 1975).

The selector gene model assumes that when selector genes remain off (as in anterior dorsal notum in Figure 2D) a ground state of development ("developmental sink": Garcia-Bellido, 1975) is attained. Turning a selector gene on causes repression of the sink pathway (Garcia-Bellido, 1977) and a new developmental pathway is followed. Thus, mutation of a selector gene would cause an entire compartment or group of compartments to follow the wrong developmental pathway.

Garcia-Bellido (1975) proposed that the most likely candidates for selector genes were those loci which, when mutant, caused homoeotic transformations. Bateson (1894) used the term homoeotic to describe mutations which transformed one structure in a meristic series to another element of the series. Garcia-Bellido (1975) applied the term both to mutations which transformed one segment to another, and those which transformed a compartment to another compartment (of the same or a different segment). In particular, he suggested that the gene responsible for division of the wing into anterior and posterior compartments was engrailed (en, 2-62.0; Eker, 1929). When engrailed$^+$ is on, cells are supposed to form posterior wing; when en$^+$ is off,

they form anterior. Mutation of *engrailed* would result in the formation of two anterior compartments in the wing and no posterior. This hypothesis has also been favoured by Lawrence and Morata (1976), who have, in addition, suggested that *engrailed* might play a similar role in many or all segments (Morata and Lawrence, 1978, 1979; Lawrence, Struhl and Morata, 1979; Lawrence and Struhl, 1982).

Several aspects of the transformation caused by the original *engrailed* allele (*en*^{1}, a stable, spontaneous mutation) leant plausibility to this explanation. The wings of *en*^{1} homozygotes show some anterior structures in the posterior compartment (Garcia-Bellido and Santamaria, 1972; Figure 3). Vein pattern in the posterior compartment is disrupted, although the anterior pattern is unaffected. Vein IV tends to resemble a mirror-image duplicate of anterior vein III, including the formation of sensilla campaniformia (non-bristled sensory organs). Along the posterior wing margin, the normal posterior double row bristles are mixed with heavy medial triple row bristles which are characteristic of the anterior margin in wild type flies. The distal wing blade is broadened. Proximal posterior structures are less affected; for example, the alula acquires socketed bristles resembling those of the anterior costa, but its shape is unaltered. The wing hinge is unaffected.

The question arose whether the anterior-posterior compartment boundary was still present in the wings of *engrailed* flies. Since the boundary had originally been defined by clonal analysis (Garcia-Bellido, Ripoll and Morata, 1973, 1976; Steiner, 1976), this approach was used to examine the effect of *engrailed* on boundary formation. Two types of experiments were performed (Morata and Lawrence (1975; Lawrence and Morata, 1976); first, marked clones (*multiple wing hair*, *javelin*: *mwh jv*) induced in *en*^{1}/*en*^{1} wings were examined. Although such clones clearly define the compartment boundary in wild type wings if induced after blastoderm, the clones in *engrailed* wings often crossed between anterior and posterior compartments, even when induced

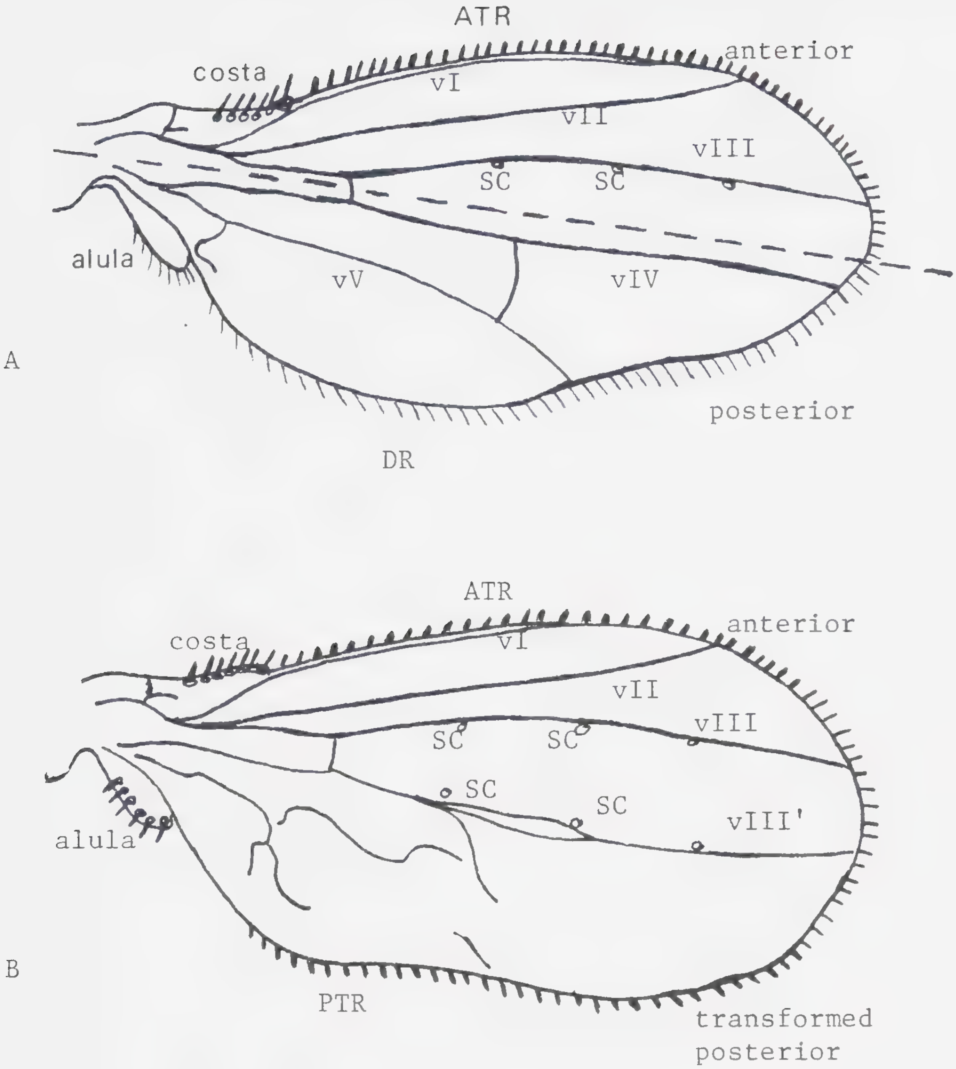


Figure 3. Wild type and *en*^{1}/*en*^{1} wings.

A. Wild type wing. Note heavy anterior triple row bristles (ATR) and fine posterior double row bristles (DR) lining margins. Costa has heavy socketed bristles, while alula has hairs. Sensilla campaniformia (SC) are found on anterior vein III (vIII), but not on posterior vein IV (vIV). Dashed line indicates anterior-posterior boundary. According to Demerec (1950) veins correspond to the following morphological designations: vI=radius 1, vII=radius 2 and 3, vIII=radius 4 and 5, vIV=media 1, vV=media 3 and 4 (Snodgrass, 1935).

B. *en*^{1}/*en*^{1} wing. Posterior double row bristles have been replaced by triple row (PTR) and socketed bristles appear on alula. Wing blade broadens distally and posterior vein pattern is disrupted. Vein IV resembles mirror-image duplicate of vein III, including appearence of sensilla campaniformia (vIII'). (After Garcia-Bellido and Santamaria, 1972; Lawrence and Morata, 1976.)

much later in development (60±12 hours AEL or 84±12 hours AEL in $M(3)i^{55}$/+ flies). This suggested that the anterior-posterior compartment boundary was not present in the wings of engrailed flies. The second approach was to make marked en^{1}/en^{1} clones in an en^{1}/+ background. These clones defined the anterior-posterior compartment boundary when they approached it from the anterior, indicating that removal of the en^{+} allele did not alter the behavior of anterior cells. However, posterior en^{1}/en^{1} clones crossed into anterior territory rather than defining the boundary, suggesting that the difference between anterior and posterior cells had been removed.

If the hypothesis that engrailed is the selector gene for the posterior compartment is correct, then the state of engrailed is responsible for all the differences between the cells of the anterior and posterior compartments. en^{+} must lead to the production of a cell surface characteristic in posterior cells which is not present in normal anterior cells. This characteristic must mediate cell sorting and give rise to a straight compartment boundary. en^{+} must also provide information required for normal differentiation of posterior structures; in its absence, posterior cells would differentiate as if they were located in the anterior compartment.

A problem with the interpretation of engrailed as a selector gene was that homozygous en^{1} flies showed only partial transformation of the posterior wing to anterior, as opposed to the complete transformation which would be expected if a selector gene had been eliminated. It was argued that this incomplete expression might be explained by postulating that en^{1} was a hypomorphic or leaky allele, i.e. retaining some en^{+} activity (Garcia-Bellido and Santamaria, 1972; Garcia-Bellido, Ripoll and Morata, 1976; Lawrence and Morata, 1976; see Muller, 1932, for description of hypomorphic mutations). If this hypothesis is correct, a mutation which completely eliminates engrailed function would be expected to cause a complete transformation of the posterior compartment of the wing into anterior.

A partial transformation of posterior to anterior is also seen in the first leg of males, which acquire a sex comb in the posterior compartment of the basitarsus in mirror image symmetry to the normal one in the anterior (Brasted, 1941; Tokunaga, 1961). In both wings and legs, the apparent plane of duplication coincides with the normal anterior-posterior boundary (Garcia-Bellido, Ripoll and Morata, 1973, 1976; Steiner, 1976).

The observation that *engrailed* appeared to have a similar effect in both wings and first legs prompted speculation that *engrailed* might act as the selector gene for the posterior compartment of every segment in which the anterior-posterior subdivision is found (Lawrence and Morata, 1976). This hypothesis is based on the observation that homologies seem to exist between corresponding positions within the structures of different segments, as shown by the changes caused by homoeotic mutations. For example, *bithorax* transforms only the anterior portion of the haltere into only the anterior compartment of the wing (see Lewis, 1978 for review). If anterior and posterior compartments in one segment were homologous to the compartments of another, it would not be unreasonable to predict that the same selector gene would be responsible for the anterior-posterior decision in every segment. The limited range of segments affected in en^1 homozygotes could be explained by again assuming that en^1 is a hypomorphic allele. Thus a null allele of *engrailed* might be expected to cause not only complete transformations of wing and leg, but also transformations of many other segments. Analysis of the effects of en^1 and new *engrailed* alleles has uncovered effects in all thoracic segments (Lawrence, Struhl and Morata, 1979; Lawrence and Struhl, 1982), the head (Morata and Lawrence, 1978, 1979), the genitalia (Curry, 1941; Epper and Sanchez, personal communication) and the abdomen (Kornberg, 1981b).

Abnormalities reported for en^1/en^1 flies included alteration of the bristle

pattern of all three legs (Lawrence, Struhl and Morata, 1979). Penetrance and expressivity of this abnormality vary and may be affected by genetic background. Lawrence et al. (1979) have defined the anterior-posterior compartment boundaries in the tarsi, and find that the effects of en^1 are confined to the posterior compartment. The major pattern alteration caused by engrailed is to increase the number of bristles in the posterior compartment. Since they find that the normal number of bristles in the posterior compartment is higher than in the anterior (92 in the posterior vs 86 in the anterior for the second leg), a further increase in the number of posterior bristles does not immediately suggest a posterior to anterior transformation. However, this is how it has been interpreted (Lawrence, Struhl and Morata, 1979). These authors do not report the effects of engrailed on the anterior-posterior compartment boundary in the tarsi.

Curry (1941) reported malformation and rotation of the male genitalia in en^1/en^1. The expression of this trait was also highly variable and dependent on background modifiers. Although homozygous fertile stocks could be made in some genetic backgrounds, other backgrounds gave rise to abnormality and sterility of all en^1/en^1 males. In some extreme cases, the entire penile apparatus was absent.

More recently, a number of other engrailed alleles have been examined. In a screen for embryonic lethal mutations, Nusslein-Volhard and Wieschaus (1980) recovered six EMS-induced mutations which show weak engrailed-like wing abnormalities when heterozygous with en^1. All homozygous and trans-heterozygous combinations of these six alleles cause a deletion of the posterior region of even numbered segments in the embryo, as well as an alteration of the anterior margin and adjacent cuticle of every segment.

Kornberg (1981a) induced 58 lethal mutations classified as engrailed alleles on the basis of their failure to complement en^1 with respect to wing phenotype. Some of

these embryonic lethals proved to be chromosomal aberrations with breakpoints close to 48A1-2. When these were heterozygous with en^1, they caused wing phenotypes intermediate between that of en^1 homozygotes, and that of en^1 over the cytologically normal lethal alleles.

All of Kornberg's engrailed lethals fall into the same complementation group, and with two exceptions, all tested trans-heterozygous combinations among these lethals caused an aberrant pattern of embryonic segmentation. The exceptional alleles, en^{LA3} and en^{LA9}, survived to adult stage over one or more of the other lethal alleles, and caused weak wing abnormalities. In the lethal embryos, denticle belts were disoriented and abnormally placed. Abnormalities described as fusion of adjacent pairs of denticle belts (i.e. of adjacent segments) frequently occurred. Kornberg tested one lethal rearrangement, en^{C2}, over a synthetic deficiency of the engrailed region (generated by the segmental aneuploidy technique of Lindsley et al., 1972). This genotype could cause fusion of up to four adjacent belts of denticles. The precise pattern of segmental abnormality varied both within and between genotypes.

Kornberg (1981a) suggests that the embryonic abnormality results from the absence of engrailed in previously unrecognized posterior compartments in the embryo, and that engrailed functions in the posterior compartment of every segment. The extreme embryonic abnormalities were surprising however, in view of the relatively weak effect of the engrailed lethals in adult wings when trans-heterozygous with en^1. This prompted examination of the lethal alleles in other adult segments, which was pursued in two ways: by examining viable trans-heterozygous combinations, and by clonal analysis.

en^{C2} (also called en^2) has been reported to affect structures derived from the eye-antennal disc (Morata and Lawrence, 1978, 1979). Although en^{C2} is homozygous

lethal, *en*^{1}/*en*^{C2} flies survive to adult stage and show relatively strong wing abnormalities. Head abnormalities occur with low penetrance; the most frequent is a duplication of the second and third antennal segments and the arista, often in conjunction with absence of other structures.

Morata and Lawrence (1979) observed a compartment boundary in the derivatives of the antennal disc by clonal analysis. Although this boundary is apparently laid down much later than the anterior-posterior compartment boundaries in the thoracic discs (72-84 hours AEL [*Minute* time] as opposed to blastoderm for the thoracic discs), they believe that it does represent the division into anterior and posterior compartments. This hypothesis is based on examination of the heads of *spineless-aristapedia* (*ss*^{a}) mutants, which have arista and distal antenna transformed into distal second leg (Balkaschina, 1929). It was hoped that *ss*^{a} would reveal any homology between compartments in the leg and those in the antenna. Somatic clones in the heads of such mutant flies could extend from the proximal antennal into the distal leg tissue. If induced after 84 hours AEL however, the clones did not cross the anterior-posterior compartment boundary in either antennal or leg tissue. Clones in the anterior leg only extended into the region of the antenna which Morata and Lawrence designate anterior; the same applied to posterior clones. Thus they believe that the late compartment boundary in the head actually corresponds to the anterior-posterior boundaries in the thorax.

In their examination of *en*^{1}/*en*^{C2} head abnormalities, Morata and Lawrence (1978, 1979) found that duplicated structures are generally confined to the anterior compartment, and that posterior structures may be absent. They contend that this represents transformation of posterior compartment derivatives into duplicated anterior compartments, similar to what is seen in *en*^{1}/*en*^{1} wings.

Effects of several *engrailed* alleles have also been reported for male and female

genitalia (Epper, 1980; Epper and Sanchez, personal communication; Epper and Kornberg, personal communication). All viable combinations of en^{1}, en^{C2}, and en^{LA3} were examined, with the interesting result that the genotypes producing the most extreme genital abnormalities are those with the weakest posterior to anterior transformations in the wing and first leg. Abnormalities are limited to absence or reduction of normal structures; mirror image duplications are not found. These investigators have suggested that each of the three terminal segment primordia (female genitalia, male genitalia, and analia; see Nothiger et al., 1977) comprises an anterior and a posterior compartment, and that *engrailed* affects only the posterior in each segment. Such compartmental divisions are not consistent with the findings of Wieschaus and Nothiger (1982), who suggest that the female genitalia and part of the male genitalia comprise an anterior compartment, while the penile apparatus and the analia are posterior. The clonal analysis of intersexual flies which would be required to distinguish these hypotheses has not yet been provided.

In a clonal analysis study, Kornberg (1981a) found that three cytologically normal lethal alleles, en^{LA4}, en^{LA7}, and en^{LA10}, behaved in wing clones much like en^{1}. There was no evidence for reduction in size or number of such homozygous clones in the wing, suggesting that these alleles are not cell lethal. Kornberg (1981b) also reported the effects of en^{LA4} clones in the first and second abdominal tergites. He identified a dividing line which he believes to be the anterior-posterior compartment boundary in the first abdominal segment. Clones which were en^{+} respected this boundary from both anterior and posterior; en^{LA4} clones (in $en^{LA4}/+$ background) respected the boundary when they approached it from the anterior, but clones originating in the posterior compartment crossed into the anterior. In addition, en^{LA4} clones in the posterior of the first abdominal segment crossed the segment boundary into the second abdominal segment. Kornberg suggested that *engrailed*$^{+}$ was

required not only to maintain the compartment boundary within a segment, but to maintain the boundaries between segments. According to this model, the differences conferred by *engrailed* on posterior cells would prevent them from mixing with the anterior cells of the next segment. This model was used to explain both the embryonic pattern abnormalities and the behavior of clones in the adult abdomen.

Lawrence and Struhl (1982) extended the clonal analysis to other segments, using the lethal alleles en^{IK} and en^{IO} of Nusslein-Volhard and Wieschaus (1980). The anterior compartments are never affected by clones of these mutations, although homozygous clones from the posterior compartment (induced at 48 hours AEL in *Minute* background) sometimes transgress the compartment boundary in the wings, proboscis and legs. Wing clones were again similar to clones of en^{1}. Posterior proboscis clones were recovered in reduced frequency, suggesting some cell lethality; those that were recovered sometimes caused abnormal bristle pattern. Posterior leg clones were markedly abnormal, often causing gross enlargements of the leg and increases in bristle number. However, when clones occurred in the male first leg, they did not produce an ectopic sex comb as is found in the en^{1}/en^{1} adult. Both posterior and anterior antennal clones were normal in appearance, size, and number. These clones crossed freely between anterior and posterior, but this result is expected, since the boundary forms later in the antenna. There was a shortfall of humeral clones, but those found appeared normal; it is not known if a compartment boundary exists in the humerus. Clones in the male genitalia and analia were small, and often associated with missing or abnormal structures; genital clones were found at a lower frequency than expected. In the female analia, *en*-lethal clones did not differ from the en^{+} controls. Due to difficulty in scoring the markers involved, no conclusions could be drawn about the *en*-lethal clones in the female genitalia.

Although several *engrailed* alleles have now been characterized, there have been

no reports of the effects in adult tissues of deleting the locus. While it is possible to obtain amorphic alleles of a locus without deleting the gene entirely, deletion of the locus is the best way to be certain there is no residual gene activity. Characterization of alleles which have reduced or altered gene activity may be misleading as to the function of the wild type gene. The embryonic lethal phenotype may be the result of an amorphic condition at the *engrailed* locus; however, it is difficult to see how this interpretation is consistent with the relatively weak effect of the lethal alleles in the wing. The observation that different *engrailed* alleles may produce very different degrees of expression among segments, with no one allele being most extreme in all segments, makes it impossible to determine which allele, if any, is an amorph.

Analysis of *en*^{1} led to the hypothesis that *engrailed* was a selector gene for posterior wing, and that its absence caused a transformation of the posterior compartment into mirror image anterior. This hypothesis has also been extrapolated to other segments. Analysis of several other alleles has produced some observations which support the hypothesis, but also others which clearly do not. Thus it may be possible to interpret the effects of *engrailed* in more than one way by examining different subsets of abnormalities produced by different alleles.

In order to observe the effects of absence of *engrailed*$^{+}$, it is crucial that a deletion of the locus be characterized. A study of the interactions of other alleles with the deletion would also help clarify the character of those alleles. For this reason, a deletion of the *engrailed* locus was constructed and examined.

MATERIALS AND METHODS

Mutations and chromosomes

The *engrailed* mutations and other mutant chromosomes used in these studies are described in Table 1. Isolation of the *engrailed* deficiencies and construction of recombinant chromosomes and stocks are described in the Results. Unless otherwise indicated, references for all mutations can be found in Lindsley and Grell (1968).

Culture conditions

Flies were grown on standard killed yeast medium, in half pint bottles containing about 50 ml of medium or vials containing about 8 ml of medium. The medium contained 1.5 grams of agar, 10 grams of sucrose, 10 grams of brewers yeast, 1 ml of propionic acid, and 10 micrograms of chloramphenicol per 100 ml of distilled water (Nash and Bell, 1968).

Stocks were maintained at room temperature, about 22° C. All crosses were performed at 22° C, unless stated otherwise. For temperature sensitivity studies, females were allowed to lay eggs at 25° for 24 hours. Eggs were then transferred to controlled temperature incubators at the desired temperature.

Matings were performed in glass vials. Parents were transferred to fresh vials or bottles after females began laying a reasonable number of eggs. For some experiments, the parents were transferred to fresh food several times at 1 to 4 day intervals in order to obtain the maximal numbers of progeny.

Preparation and examination of flies

Flies were prepared for examination by heating in 0.5 N NaOH until eye pigment disappeared, washing for several minutes in distilled water, then storing in a mixture of 3 parts 95% ethanol; 1 part glycerol. Flies were then dissected in water and mounted between coverslips in Gurr's water mounting medium. Wing vein abnormalities,

posterior triple row bristles, posterior sex comb teeth, sensilla trichodea (in transformed wing tissue and in metathoracic halteres) were scored in mounted flies under a compound microscope. Wing shape was determined using a grid eyepiece for the compound microscope to measure width and length. Whole flies were placed in alcohol after heating in NaOH and examined under a dissecting microscope for posterior claw abnormalities. Wing to haltere transformation was scored under a dissecting microscope. Antennal abnormalities for Table 25 were scored in mounted flies. Head abnormalities following irradiation were scored in whole flies under a dissecting microscope.

Salivary gland chromosome cytology

Larvae were grown in uncrowded conditions at 22° C and large third instar females chosen for cytology. Females were distinguished from males by examining the paired gonads which lie laterally in the posterior third of the body cavity. Ovaries are much smaller and more spherical than testes (Demerec, 1950). Salivary glands were removed in 45% acetic acid, transferred to aceto-orcein stain for 1 to 2 minutes, then squashed in acetic acid between a slide and coverslip. Preparations were examined under a compound microscope using phase contrast lenses, and cytological locations of engrailed aberrations determined according to the maps of Bridges and Bridges (1939).

Head abnormalities following irradiation

Flies for this study were grown at 22° C on corn meal medium. This medium consists of 0.45% agar, 5.0% dextrose, 2.5% sucrose, 8.3% corn meal, 1.5% dried yeast, 0.06% phosphoric acid and 0.4% propionic acid (Lewis, 1960). Females were allowed to lay eggs in half pint split bottles for 24 hours. Larvae were irradiated for 2.5 minutes (20 cm, 50 mV). For the series of time points before puparium forma-

tion, newly formed pupae were collected at 12 hour intervals and transferred to fresh vials.

Construction of mutant combinations with Bithorax-Complex alleles

A stock was established carrying *en30*/*SM5*;*sbd*^{2}*bx*^{3}*pbx*/*TM1*. For the data shown in Table 14, females of this stock were crossed to *en28*/+;*pbx*/+ males. For Table 15, females were crossed to *en28*/+;*mwh* *jv* *sbd*^{2}*bx*^{3}*red*/+ males. In each case, all *en28*/*en30* progeny were collected so that flies homozygous for *bx*^{3} or *pbx* could be compared to their non-homozygous sibs. Non-recombinant *en28* and *en30* chromosomes were used for this study.

Table 1. Mutations and chromosomes used.

A. engrailed mutations	Reference
en^1	Eker(1929)
en28 (Df 47B3 to 47B9-14,In 47B9-14 to 48A1-2, Df 48A1-2 to 48B-C1)	Russell and Eberlein(1979); this study
en30 (Df 48A3-4 to 48C6-8)	Russell and Eberlein(1979); this study
Df(2)enA (Df 47D3 to 48B2-5)	D. Gubb(in preparation)
Df(2)enB (Df 47E3-6 to 48A4)	D. Gubb(in preparation)
engrailed lethals: IM99, IO34 (from Nusslein-Volhard and Wieschaus)	Nusslein-Volhard and Wieschaus(1980)

B. Special en^1 chromosomes	Source and reference^1
cn en^1 (cn=cinnabar)	
pwn en^1 (pwn=pawn)	from P. Lawrence (see Garcia-Bellido and Dapena(1973) for pwn)
lt stw en^1	
In(2L)Cy, cn Cy en^1 (Cy=Curly)	from D. Gubb

C. Chromosomes for construction of engrailed recombinants and mapping	
cn vg (vg=vestigial)	
pr pwn cn sp (pr=purple pwn=pawn)	
al dp b pr cn vg c a px bw mr sp (al=aristaless dp=dumpy b=black c=curved a=arc px=plexus bw=brown mr=morula)	
Sp Bl L Bc Pu Pin (Sp=Sternopleural Bl=Bristle L-Lobe Bc=Black cells Pu=Punch)	see Grell(1969) for Bc

D. Chromosomes for Bithorax-Complex interaction studies
mwh jv sbd^2 bx^3 red(mwh=multiple wing hair jv=javelin sbd=stubbloid bx=bithorax)
pbx (postbithorax)
sbd^2 bx^3 pbx

Table 1. (continued)

E. Chromosomes for isolation of engrailed deficiencies

b Tft (Tft=Tuft)
cn en^1

F. Balancers

Second chromosome

SM5 (In(2LR)SM5, al^2 Cy lt^v cn^2 sp^2)

CyO (In(2LR)O, dp^{1vI} Cy pr cn^2)

Third chromosome

TM1 (In(2LR)TM1, Me ri sbd^2; Me=Moire, ri=radius incompletus)

TM1 red (as above, also carrying red)

---

1. Unless otherwise indicated, references for all mutations and chromosomes can be found in Lindsley and Grell (1968). Sources of chromosomes are Bowling Green and Cal Tech Stock Centers.

RESULTS

Isolation of *engrailed* deficiencies

Mutations of *engrailed* were induced with X-irradiation because X-rays are known to cause chromosome breaks and deletions. Figure 4 shows the mating scheme employed to isolate new mutant alleles. Potential mutations were identified by the wing phenotype produced over en^1. Because *engrailed* homozygotes are usually sterile, an F_2 screen was carried out. Out of 2158 pair matings, two were found in which Cy^+ Tft^+ flies showed the *engrailed* wing abnormalities. F_2 *Cy* sibs were mated to establish stocks of these two presumptive new *engrailed* mutations. Among the Cy^+ F3 progeny from the first mutant stock, both *engrailed* and wild type flies were found. Subsequent progeny testing of single F_3 *Cy* flies produced one of two results:

1.(Figure 5A) When F_3 *Cy* flies were pair mated, a balanced stock could be made which gave only *Cy* progeny. When crossed to en^1/*SM5*, flies from these lines produced only progeny with either *Cy* or *engrailed* wing phenotype. This mutant chromosome was designated *en28*. The *en28* chromosome was examined cytologically and shown to be a complex rearrangement (Df 47B3 to 47B9-14, In 47B9-14 to 48A1-2, Df 48A1-2 to 48B-C1, Figure 6; B. Baker, personal communication and my results). It does not survive over lethal alleles of *engrailed*; therefore it deletes the locus which I shall refer to as the "*engrailed* vital locus".

2.(Figure 5B) A balanced stock could be made from F_3 *Cy* flies, but when crossed to en^1/*SM5*, both *engrailed* and wild type wings were found among the non-*Cy* progeny. Further crosses showed the wild type flies to be *en28*/en^1, since a single fly could segregate both alleles. When such flies were crossed to flies carrying marked first and third chromosomes, a factor on the third chromosome was found to be responsible for the suppression of the *engrailed* abnormalities. This factor was mapped to 3L, distal to *hairy* (3-26.5); cytologically it is an insertion of two bands into 62C1. The insertion suppresses all *engrailed* effects,

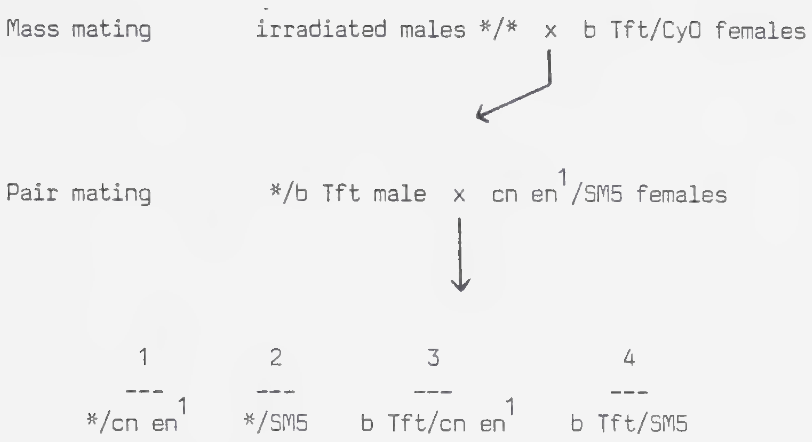


Figure 4. Isolation of new *engrailed* mutations.

Males were irradiated with a Colbalt 60 source(4000r) and crossed as outlined. Progeny of the pair matings were examined for the presence of engrailed-like wings(class 1 progeny). If such wings were observed, class 2 sibs were mated to make a stock. *b* = *black* *Tft* = *Tuft*, a dominant mutation affecting thoracic bristles, used to differentiate between classes 1 and 3. CyO, SM5 = second chromosome balancers carrying the dominant *Curly* wing mutation and the recessive *cinnabar* (*cn*) eye. Presence of *cinnabar* eyes was used to control for false positives caused by non-virgin *cn en*^{1} females; newly induced *engrailed* mutations should be on a *cn*$^+$ chromosome.

Figure 5. Identification of the insertion portion of the *en28* transposition.

A. Class 1

Phenotype observed	Genotype inferred
Cy x Cy F3 sibs	Df(2)en28/SM5 x Df(2)en28/SM5
↓	↓
only Cy	Df(2)en28/SM5
Cy x cn en^1/SM5 outcross	Df(2)en28/SM5 x cn en^1/SM5
↓	↓
Cy	Df(2)en28/SM5
cn Cy	cn en^1/SM5
en	Df(2)en28/cn en^1

B. Class 2

Phenotype observed	Genotype inferred
Cy x Cy F3 sibs	Df(2)en28/SM5; Dp(3)en28/+ x Df(2)en28/SM5;(+/+)
↓	↓
only Cy	Df(2)en28/SM5; Dp(3)en28/+ (or +/+)
Cy x cn en^1/SM5 outcross	Df(2)en28/SM5; Dp(3)en28/+ x cn en^1/SM5
↓	↓
Cy	Df(2)en28/SM5; Dp(3)en28/+ (or +/+)
cn Cy	cn en^1/SM5; Dp(3)en28/+ (or +/+)
en	Df(2)en28/cn en^1; +/+
Cy$^+$ cn$^+$ en$^+$	Df(2)en28/cn en^1; Dp(3)en28/+
Cy$^+$ cn$^+$ en$^+$ F4 x cn en^1/SM5	Df(2)en28/cn en^1; Dp(3)en28/+ x cn en^1/SM5
↓	↓
Cy	Df(2)en28/SM5; Dp(3)en28/+ (or +/+)
cn Cy	cn en^1/SM5; Dp(3)en28/+
cn en	cn en^1/cn en^1; +/+
cn$^+$ en	Df(2)en28/cn en^1; +/+
cn en$^+$	cn en^1/cn en^1; Dp(3)en28/+
Cy$^+$ cn$^+$ en$^+$	Df(2)en28/cn en^1; Dp(3)en28/+

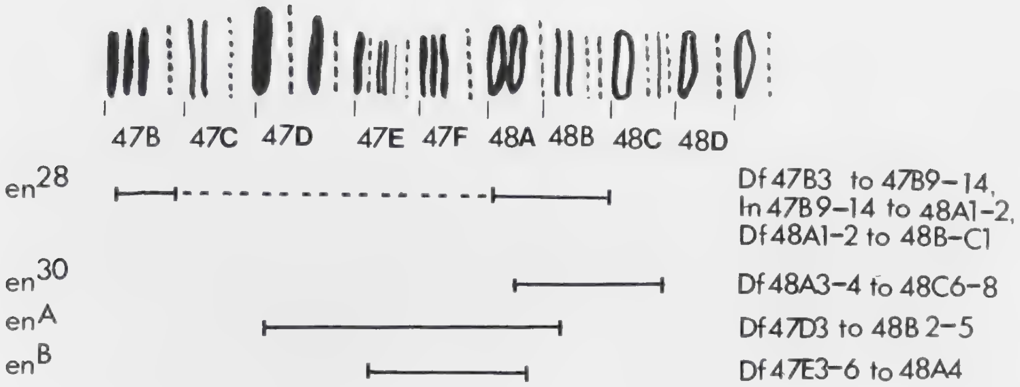


Figure 6. Salivary gland cytology. Diagram represents the banding pattern of the right arm of the second chromosome. Solid lines represent deleted regions; dashed lines represent inverted regions. The locations of *en28* and *en30* are shown, as well as the locations of the deficiencies *enA* and *enB* (D. Gubb, personal communication). This result is consistent with the results of Kornberg (1981a) who places *engrailed* in 48A.

including the lethality of engrailed lethal point mutations. It does not suppress the lethality causes by homozygosis of either en28 or en30 (see below), but this may be due to other loci deficient in these deletions. The insertion is homozygous viable in engrailed or wild type background. Since both the en28 second chromosome and the insertion were recovered from a single F2 fly, it is probable that the engrailed locus was deleted from 2R and inserted into 3L.

The second pair mating produced only Cy winged flies in the F_2, showing that the mutagenized chromosome carries at least one recessive lethal. Cytological examination showed this chromosome to carry a deletion of 48A3-4 to 48C6-8. This deletion was designated en30 (Figure 6).

Recombination mapping of en28 and en30 was undertaken to ensure that the wing abnormalities produced by these chromosomes over en^1 mapped to the same position as en^1 (2-62.0). Figure 7 shows the mating scheme used to map en28 and en30 against cinnabar (cn, 2-57.5) and vestigial (vg, 2-67.0). Results of these crosses are shown in Table 2. For both new mutant chromosomes, the wing abnormality maps between cn and vg and shrinks the map distance separating these markers, as expected of a deletion.

After stocks of en28/SM5 and en30/SM5 were established, flies from these stocks were mated to each other to determine whether a fly of the genotype en28/en30 would survive. Since SM5 is homozygous lethal, the expected ratio of progeny from such a cross is 1 en30/SM5: 1 en28/SM5: 1 en28/en30, assuming engrailed does not affect viability. Phenotypically, this means there are two Cy flies expected for every non-Cy (en28/en30) fly. Non-Cy progeny with engrailed-like wings were recovered at frequencies of up to 20% of that expected. This high survival of en28/en30 flies was surprising since they apparently lack the large chromosomal region 48A3-4 to 48B-C1 (see Figure 6). However, several other observations suggested an explanation for this surprising result.

A. cn^+ en* vg^+/SM5 x cn en^+ vg/SM5

cn^+ en* vg^+

1 2 females x cn en^1 vg/SM5 males

cn en^+ vg

Non-SM5 progeny (Cy^+):

Parental: cn en^+ vg = cn en^+ vg/cn en^1 vg

cn^+ en vg^+ = cn^+ en* vg^+/cn en^1 vg

Single recombinants:

Region 1 cn en vg^+ = cn en* vg^+/cn en^1 vg

Region 2 cn^+ en vg = cn^+ en* vg/cn en^1 vg

Double recombinants:

cn en vg = cn en* vg/cn en^1 vg

cn^+ en^+ vg^+ = cn^+ en^+ vg^+/cn en^1 vg

B. cn 7.5 en^1 6.2 vg

cn 8.0 en30 4.2 vg

cn 4.0 en28 5.4 vg

Figure 7. Mapping of the new *engrailed* mutations.
A) Crosses to obtain maps.
B) Map distances for *en^1*, *en28*, and *en30*. Expected distances are cn-en: 4.5, en-vg: 5.0 (Lindsley and Grell, 1968).

Table 2. Recombination mapping of <u>en28</u> and <u>en30</u>.

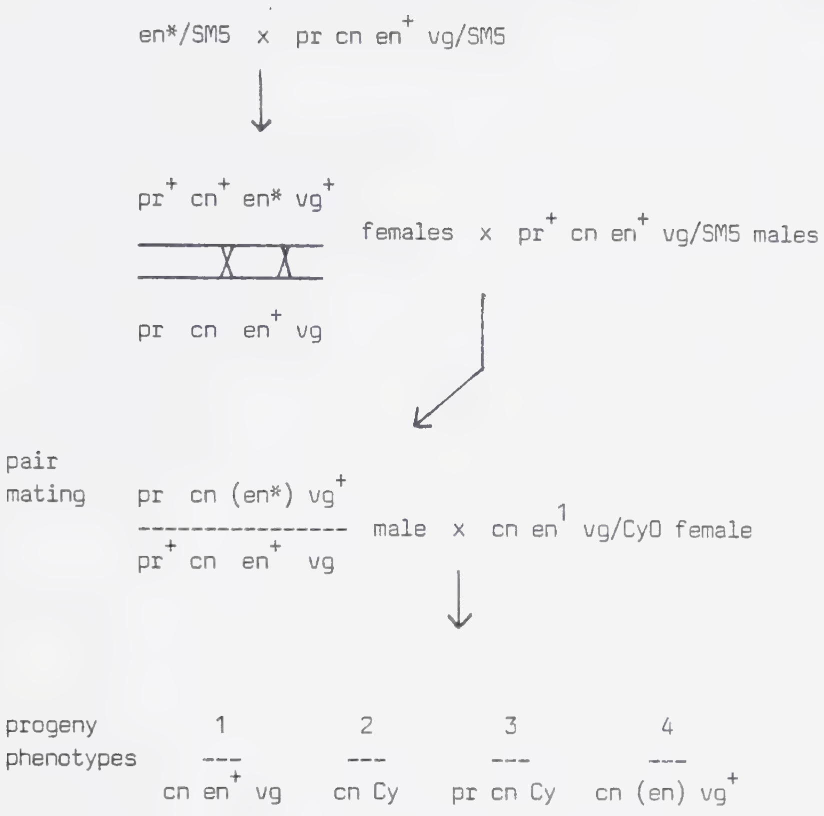
Progeny class[1]		en^1	en28	en30
parental:				
	cn + vg	777	396	717
	+ en +	933	319	857
single:				
	cn en +	64	13	57
	+ + vg	77	14	77
	cn + +	62	29	36
	+ en vg	54	9	31
double:				
	cn en vg	4	2	2
	+ + +	3	2	6
total		1984	784	1783

1. See figure 7 for outline of recombination crosses.

A series of crosses was undertaken to recover the en28 and en30 aberrations after recombination to a non-irradiated, marked second chromosome (Figure 8). This was to ensure that any other mutations induced simultaneously with the engrailed aberrations would be removed from the chromosome, and to provide a variety of genetic backgrounds in which the effects of en28 and en30 could be compared. Figure 8 shows an example of a proximal recombination event and the resultant chromosome. When such a recombination event occurred between cn and en28, the resultant chromosome was still viable over en30. However, the equivalent event between cn and en30 produced a chromosome which no longer survived over en28. This observation suggested that a region on the en30 chromosome proximal to en30 on 2R or on 2L was required for survival of en28/en30. I considered the possibility that some of the material deleted from region 48 in en30 had been transposed elsewhere on the second chromosome.

Cytological examination of the en30 chromosome did not reveal any obvious insertion of material, suggesting that the transposition was either too small to be detected, or had inserted into the centric heterochromatin. Two series of experiments were undertaken to locate the chromosomal region required for survival of the en30 chromosome over other engrailed region aberrations.

The first involved comparing the survival of the original en30 chromosome with proximal recombinants. Several en30 chromosomes were examined after recombination between cn and en30 (see Figure 8). Table 3 shows a typical example of survival of the original en30 chromosome and one recombinant. Also shown is the frequency with which en28/en30 progeny can be obtained when employing either a recombinant en28 or en30 chromosome; in both cases, recombination involved the same marked second chromosome. The original and several recombinants of the en30 chromosome were also crossed to a larger deficiency of the engrailed region, Df(2)enB (D. Gubb, personal communication; see Figure 6 for cytology). Table 3 shows that this deficiency behaves much



genotypes: 1) cn en^+ vg/cn en^1 vg 2)cn en^+ vg/CyO

3) pr cn (en*) vg^+/CyO

4) pr cn (en*) vg^+/pr^+ cn en^1 vg

Figure 8. Isolation of engrailed chromosomes after recombination between cinnabar and engrailed.

en* represents one of en^1, en28, or en30. If the recombinant chromosome carries en*, class 4 progeny will have engrailed wings. The chromosome can be recovered in class 3 sibs. CyO is a balancer carrying cinnabar (cn) and purple (pr).

Table 3. Effect of recombination of *en30* chromosome on *en28*/*en30* viability.

Cross	# SM5 progeny	# *en/en* progeny	frequency
non-recombinant *en28*/SM5 x non-recombinant *en30*/SM5	1106	116	0.10
pr *pwn* *cn* *en28*/SM5 x non-recombinant *en30*/SM5	921	77	0.08
pr *pwn* *cn* *en30*/SM5 x non-recombinant *en28*/SM5	557	0	0
enB/CyO x non-recombinant *en30*/SM5	250	37	0.13
enB/CyO x recombinant *en30*/SM5	1400	0	0

1. Crosses were done reciprocally and data pooled.
2. Frequency is number of *en/en* progeny out of total progeny.
   Expected frequency from these crosses is 0.33.
3. Data are pooled for results of crosses involving *enB* and two recombinant *en30* chromosomes. Both *en30* chromosomes were derived from recombination events between *cn* and *en30*, but two different chromosomal backgrounds were involved.

like *en28* regarding survival over the original and recombinant *en30* chromosomes.

The second approach involved recombination of either *en28* or *en30* chromosomes with a second chromosome carrying six dominant markers (Figure 9). Recovery of rec-*en28*/*en30* and rec-*en30*/*en28* was compared after recombination had occurred in each of the six regions shown in Figure 9. *engrailed* maps between *Bristle* (*Bl*) and *Lobe* (*L*), *Bl* being located on the other side of the centromere. If a transposition to the centromere had occurred, many of the recombination events between *Bl* and *en30* (region 2b) would separate the *en30* deletion from the insertion. Such recombinant chromosomes would not be expected to survive over *en28*. Events in the same region involving the *en28* chromosome should produce *Bl* *en28* recombinants which do survive over *en30*. Recovery of single recombination events in any other region of the chromosome would not depend on whether the *en28* or the *en30* chromosome was recombinant, since other events would not separate the *en30* deletion from the putative insertion. Thus the frequency of *Bl* *en30*/*en28* progeny among the total rec-*en30*/*en28* progeny should be lower than the frequency of *Bl* *en28*/*en30* among the total rec-*en28*/*en30* progeny. The results in Table 4 show that this expectation is met. Among the recombinant *en28* chromosomes, 6.5% were recombinant in the *Bl*-*en28* region; among the recombinant *en30* chromosomes recovered, only 2.8% were recombinant between *Bl* and *en30*. Thus recovery of recombinants in this region was reduced by 57%. A contingency X^2 test (Table 4) shows this difference to be significant at the .025 probability level, whereas recovery of recombinants in any other region did not differ significantly between the *en28* and *en30* chromosomes.

These results confirm that a region between *Bl* and *en* on the *en30* chromosome is necessary for the survival of *en30*/*en28* flies, and are compatible with the hypothesis that some of the material deleted from region 48 has been inserted in proximal 2R or 2L in the *en30* chromosome. The recombinant *en30* chromosomes still survive over

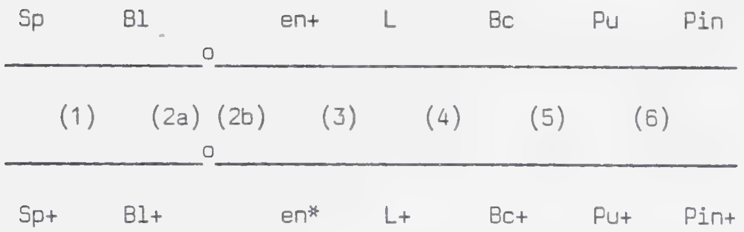


Figure 9. Multiply marked second chromosome used for recombination of en28 and en30.

Table 4. Recovery of en28/en30 progeny carrying recombinant en28 or en30 chromosomes.

Region of recombination[1]	Recombinant en28 chromosomes	Recombinant en30 chromosomes	Homogeneity X^2	p value[2]
Sp - Bl (1)	5	12	0.002	> 0.95
Bl - en (2)	13	13	5.076	< 0.025
en - L (3)	8	19	0.002	> 0.95
L - Bc (4)	18	30	1.343	> 0.05
Bc - Pu (5)	23	67	1.042	> 0.05
Pu - Pin (6)	21	54	0.204	> 0.05
non-recombinant	113	272		
total	201	467		

1. See Figure 9 for recombination regions.
2. p value gives the probability that the recovery of recombinants in a given region is not significantly different depending on which chromosome is undergoing recombination.

engrailed lethals and *en*^{1}, and such flies are phenotypically indistinguishable from those carrying the non-recombined *en30* chromosome. This suggests that the transposed material includes another vital locus which is also deleted in *en28* and *enB*, but not the *engrailed* locus itself. The locus to which the *engrailed* lethality is ascribed must still be present at the original site on the *en30* chromosome.

Kornberg (1981a) places the *engrailed* locus in 48A; the above results are consistent with this location. Since embryos carrying *en28* over the *engrailed* lethals do not survive, the vital locus must be deleted or broken in the *en28* chromosome. *en30* does not affect the vital locus, suggesting that the most probable location of *engrailed* lethality is between 48A1-2 and 48A3-4 (Figure 6).

Since *en28* has a breakpoint in 48A, the question arises as to whether it completely inactivates or merely reduces the activity of the *engrailed* locus. Two larger deficiencies of the *engrailed* region, *enA* and *enB* (kindly provided by D. Gubb) were used in phenotypic comparison to settle this question. The relative positions of all four aberrations are shown in Figure 6. *enA* and *enB* clearly remove the region of the *engrailed* vital locus and the region containing the 48A breakpoints of both *en28* and *en30*. Thus *enA* and *enB* should show the effects of completely deleting this region. All abnormalities considered in this study appeared to a similar degree in genotypes containing *en28* or those containing *enA* or *enB* (with one possible exception to be discussed below). No new abnormalities appeared with the larger deficiencies. This suggests that the *engrailed* locus is completely inactivated in *en28*.

Phenotypic effects of the new *engrailed* alleles

To learn if *en*^{1} was actually a hypomorph, it was necessary determine whether *en28* or *en30* produced a more severe phenotype than *en*^{1}. The first part of this analysis considered the abnormalities observed in *en*^{1} homozygotes. The pleiotropic effects were separated into single phenotypic characteristics to facilitate quantification. These characteristics will be referred to as Group 1 abnormalities. Genotypes considered were *en*^{1}/*en*^{1}, *en28*/*en*^{1}, *en30*/*en*^{1} and *en28*/*en30*. Within each genotype, at least four strains were employed, the genetic backgrounds of which had been randomized in the following manner:

> Recombination on the second chromosome to replace the region proximal to *cn* and distal to *vg* was as outlined in Figure 8. Four different second chromosomes were used to produce recombinants so that background would be varied. In the process of obtaining these recombinants, first, third and fourth chromosomes became randomized as outlined in Figure 10. Crosses performed to obtain an *en*/*en* genotype involved balanced *engrailed* chromosomes from two different outcrosses. Within each strain, all phenotypic characteristics were examined.

Analysis of variance was performed for each characteristic to determine which part of any difference could be ascribed to differences in *engrailed* genotype, and which part to other background factors (appendices 1-4).

Figure 11 shows the wings from wild type, *en*^{1}/*en*^{1}, *en28*/*en*^{1} and *en30*/*en*^{1}. Three elements of the transformation should be noted. First, the posterior vein pattern is aberrant in all *engrailed* genotypes. This abnormality was quantified using an arbitrary scale of 1 (wild type) to 5 (most abnormal) as shown in Figure 12. The second element to be considered is the alteration in blade shape, causing the wing to become wider and more symmetrical at the distal end. This was quantified by measuring the ratio of wing width (midway between the anterior crossvein and the distal margin,

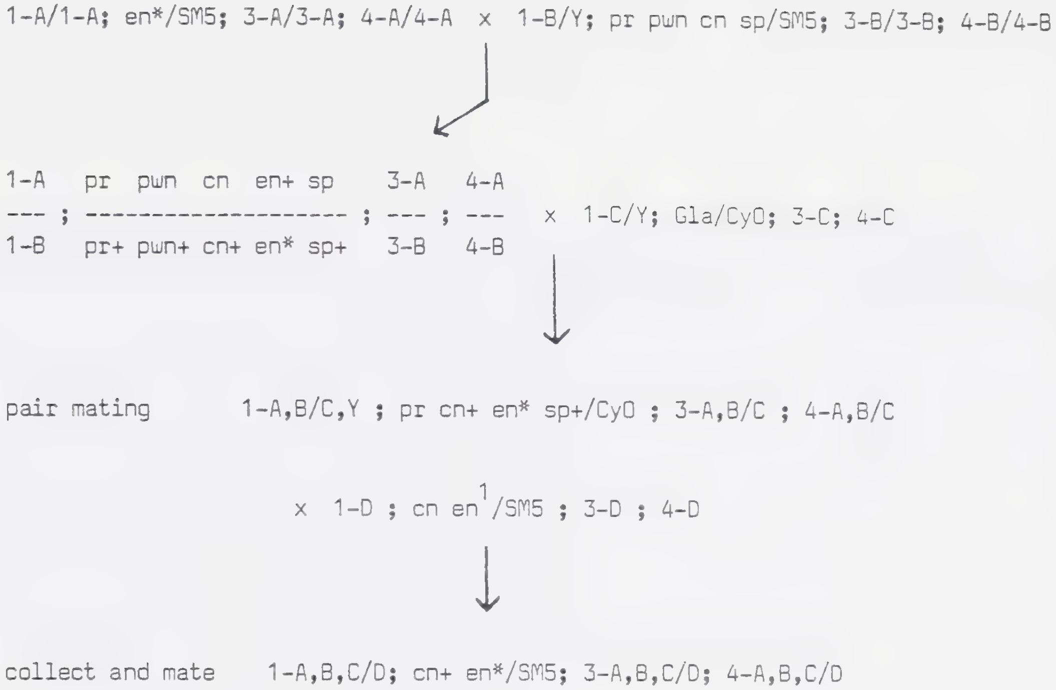


Figure 10. Generating recombinant en28 and en30 chromosomes and randomizing genetic background.

en* refers to either en28 or en30. Progeny collected to make stock will have a recombined left arm of the second chromosome and first, third and fourth chromosomes from four different backgrounds. This scheme shows the recovery of a second chromosome recombinant between purple(pr) and cinnabar(cn). This chromosome can be recognized over CyO, which carries both pr and cn. 1,3,4: first, third, and fourth chromosomes. A,B,C,D: indicate different possible chromosomes from different backgrounds. Although a given strain is depicted as carrying only one type of chromosome (e.g. 3-A/3-A) it is probable that the two homologues in each strain do differ. In addition, recombination between homologues may occur, providing more varied backgrounds.

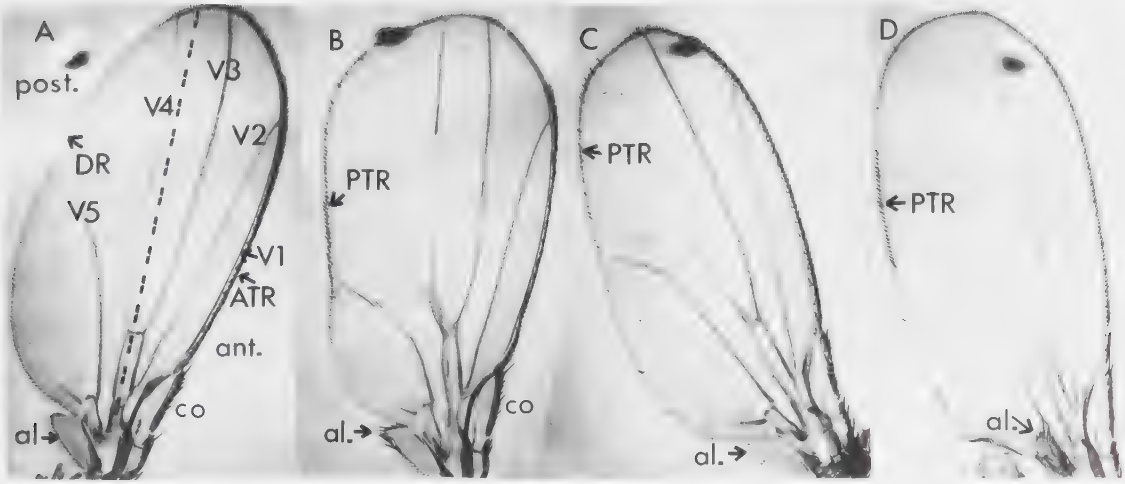


Figure 11. Wings of wild type and *engrailed* flies, bright field optics, magnification x20.

A. Wild type wing, dashed line shows normal boundary between anterior compartment (ant.) and posterior compartment (post.). V1-V5: veins 1 through 5.

B. *en*^{1}/*en*^{1}

C. *en30*/*en*^{1}

D. *en28*/*en*^{1}

Note that normal anterior triple row bristles (ATR) are present in all genotypes. Normal posterior double row hairs (DR) are replaced by posterior triple row bristles (PTR) in *engrailed* genotypes. The proximal posterior alula (al.) may acquire heavier socketed bristles (as in B,D) characteristic of the anterior costa (co), but the shape of the alula is not altered. More proximal structures remain unaffected by *engrailed*.

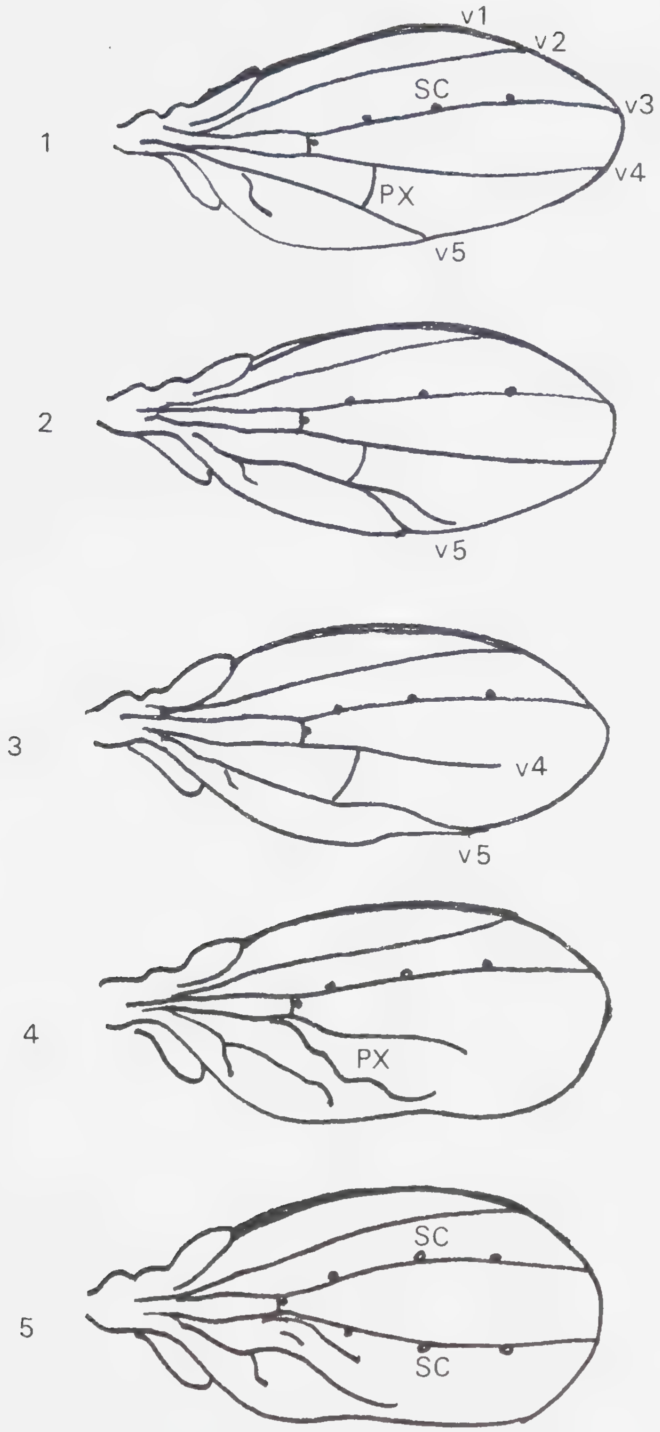


Figure 12. Scale for quantification of wing vein abnormalities. Examples for each class of abnormality, from 1 (wild type) to 5 (extreme engrailed) are shown. For details of classification, see text.
SC: sensilla campaniformia   v1-v5: veins 1 through 5
PX: posterior cross vein

perpendicular to vein 3) to length (from hinge to margin, along vein 3). The third characteristic is the number of posterior medial triple row bristles. These bristles are usually found only on the anterior margin of the wing; in *engrailed* flies they also appear along the posterior margin.

In addition to the wing abnormalities, *en*^{1}/*en*^{1} also produces a change in the first leg of the male (Brasted, 1941; Tokunaga, 1961). Sex comb teeth, normally found only in the anterior compartment, are duplicated in the posterior compartment in mirror image symmetry (Figure 13A). Number of posterior teeth may vary from a single tooth to a complete comb of ten or more teeth, and in some cases several rows or clumps of teeth appear in the posterior. For this study, the number of posterior sex comb teeth was considered.

This analysis is summarized in Tables 5 and 6. When analysis of variance was performed on all four genotypes for each characteristic, the significant differences were attributable to genotype at the *engrailed* locus rather than differences among strains, sexes, or individual flies (appendices 1-4). It should be noted however, that the difference among genotypes for a given characteristic might be attributed to a large difference between one genotype and all the other three, rather than significant differences between every pairwise combination of genotypes. For this reason, I have attempted to order the genotypes with regard to expression of each characteristic (Table 7), and have indicated as equal those pairs of genotypes whose means do not differ significantly.

It is possible to place the four abnormalities into two classes based on degree of expression in the different genotypes (Table 7, Group 1). For posterior triple row bristles (PTR) and posterior sex comb teeth (PSCT), *en*^{1} homozygotes clearly show the strongest transformation. Penetrance of PTR is significantly stronger in any genotype carrying the *en*^{1} allele than in *en28*/*en30*, suggesting an allele specific effect. All

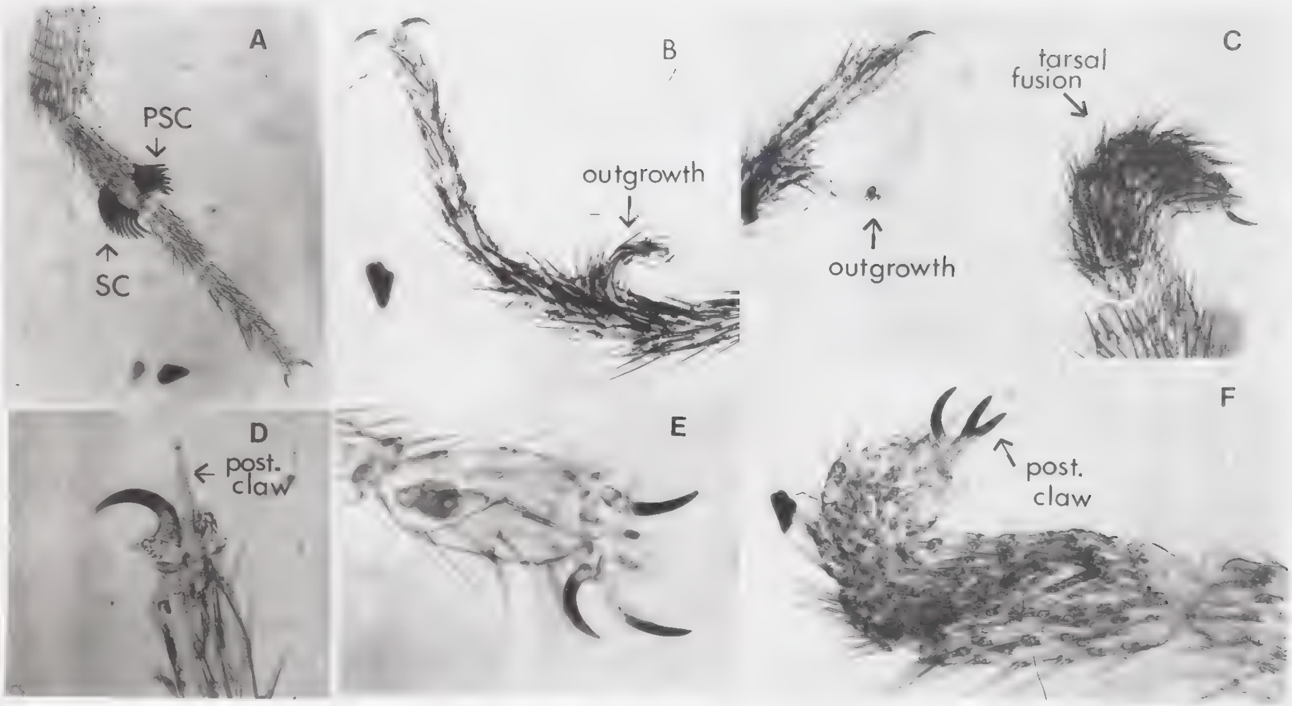


Figure 13. Leg abnormalities produced by engrailed, bright field optics.

A. magnification x60. en^1/en^1 male foreleg with mirror image duplication of the normal sex comb (SC) in the posterior compartment (PSC).
B. x90. en^1/en^1 male second leg showing an outgrowth from the posterior compartment.
C. x90. en28/en^1 female second and third legs. Note outgrowth and fusion of tarsal segments.
D. x200. en28/en30 female second leg with abnormal posterior claw (post.).
E. x210. en28/en30 female third leg with completely duplicated claw.
F. x170. en28/en^1 female third leg with partially duplicated posterior claw.

Table 5. Expressivity of abnormalities originally observed in <u>en</u>^{1}/<u>en</u>^{1}.

Genotype[1]	# Posterior medial[2,3] triple row bristles	Posterior wing vein[2,3] abnormality(scale 1 to 5)	Wing shape[2,3] % width/length	# Posterior[2,4] sex comb teeth
en^1/en^1	41.1 ± 4.5 (70)	4.7 ± 0.3 (64)	51 ± 1 (40)	8.4 ± 0.8 (90)
en28/en^1	9.1 ± 4.9 (154)	4.5 ± 0.3 (74)	49 ± 1 (46)	2.4 ± 1.3 (40)
en28/en30	0.8 ± 4.9 (188)	4.0 ± 0.4 (42)	49 ± 1 (35)	0.03 ± 1.1 (72)
en30/en^1	2.1 ± 4.9 (148)	2.5 ± 0.5 (72)	44 ± 1 (42)	0.02 ± 1.0 (52)

1. Each genotype comprises at least three strains. Analysis of variance was performed for each characteristic to show that the significant differences derived from differences between genotypes rather than differences between strains within a genotype.
2. Mean ± 95% confidence limits as determined from the analysis of variance using the following formula: 2 (error mean square/# groups in sample)$^{.5}$
3. Number of wings examined shown in parentheses.
4. Number of legs examined shown in parentheses.

Table 6. Penetrance of posterior to anterior transformation abnormalities.

Genotype	% of wings with one[1,2] or more PTR bristle	% of legs with[3] one or more PSCT
en^1/en^1	100 % (70)	100 % (90)
$en28/en^1$	84 % (154)	68 % (40)
en28/en30	23 % (188)	3 % (72)
$en30/en^1$	42 % (148)	2 % (52)

1. Number of wings or legs examined shown in parentheses.
2. PTR = posterior triple row.
3. PSCT = posterior sex comb teeth.

Table 7. Ordering of *engrailed* genotypes according to expression of abnormalities.

Phenotypic characteristics	Ordering of genotypes for[1] each characteristic	Overall order for each Group of characteristics
Group 1		
A. Posterior triple row bristles	$en^1/en^1 > en28/en^1 > en30/en^1 > en28/en30$	$en^1/en^1 > en28/en^1 > en30/en^1 \geq en28/en30$
Posterior sex comb teeth	$en^1/en^1 > en28/en^1 > en30/en^1 = en28/en30$	$en^1 > en28 > en30$
B. Posterior wing vein abnormality	$en^1/en^1 = en28/en^1 = en28/en30 > en30/en^1$	$en^1/en^1 = en28/en^1 = en28/en30 > en30/en^1$
Wing shape	$en^1/en^1 = en28/en^1 = en28/en30 > en30/en^1$	
Group 2		
Wing to haltere transformation	$en28/en30 > en28/en^1 > en^1/en^1 = en30/en^1$	$en28/en30 > en28/en^1 > en^1/en^1 = en30/en^1$
Posterior claw abnormality	$en28/en30 > en28/en^1 > en^1/en^1 = en30/en^1$	

1. Pairs of genotypes which are not significantly different for the expression of a given characteristic (according to analysis of variance) are marked as equal.
   Group 1 designates abnormalities originally observed in en^1/en^1; Group 2 designates new abnormalities observed when *engrailed* has been deleted.

results for the PTR and PSCT abnormalities are consistent with the genotypic and allelic ranking shown in Group 1A of Table 7. These two abnormalities are similar in that they both place anterior structures in the posterior compartment, and thus most clearly suggest a posterior to anterior transformation.

Posterior wing vein pattern and wing shape alterations are not as clearly indicative of a posterior to anterior transformation, and their expression does not appear to be *en*^{1} allele specific (Table 7, Group 1B). Although the mean expressivity of these abnormalities is slightly higher in *en*^{1}/*en*^{1} than any other genotype, this difference is only significant for *en*^{1}/*en*^{1} and *en30*/*en*^{1} (Table 5). It is noteworthy that both abnormalities are significantly stronger in *en28*/*en30* than *en30*/*en*^{1}, suggesting that these abnormalities are not specific to the *en*^{1} allele. The allelic ranking for these abnormalities is not clearly indicated, and their relationship to the other abnormalities will be discussed below.

New *engrailed* abnormalities

All pattern abnormalities discussed above were originally described for *en*^{1} homozygotes. I have observed several new abnormalities in adults carrying the chromosomal aberrations, which shall be designated Group 2 abnormalities.

Abnormalities which were observed in all three pairs of legs included the absence or degeneration of posterior claws and the fusion of tarsal segments. Three aspects of the leg phenotype were considered. First, fused tarsal segments were examined. When this abnormality was weak, some cases were found where the posterior part of the boundary between tarsal segments did not appear to have formed, while the anterior boundary was normal. The converse case, with normal posterior and abnormal anterior, was never observed. In cases where fusion was more extreme, both compartments were distorted (Figure 13). It was not possible to discern whether this distortion was due to a primary effect of *engrailed* in the anterior compartment, or was

caused secondarily by the extreme posterior fusion.

Outgrowths from the basitarsi and tarsi were frequently observed (Figure 13C), and in some cases duplication of claws (Figure 13E,F) and distal leg segments was observed. Similar outgrowths, including duplicated distal leg segments, were occasionally noted in some strains homozygous for $\underline{en}^1$ (Figure 13B). All outgrowths which were carefully examined appeared to originate in the posterior compartment. A similar result was noted by Fausto-Sterling and Smith-Schiess (1982) when flies homozygous for both $\underline{en}^1$ and fused alleles were examined.

The third aspect of leg abnormality was the absence or degeneration of posterior claws (Figure 13D). Most of the claws were classified as anterior or posterior by scoring their orientation on the whole fly; however, 20 legs were scored first on the whole fly then mounted for more detailed analysis. Rows of bristles were followed in the mounted leg to determine which compartment contained the abnormal claw, according to the leg compartment maps of Steiner (1976). In all cases, rigorous scoring confirmed my initial impression that the posterior claw was affected.

I also noted a homoeotic tranformation which occurred frequently in en28/en30 and rarely in en28/$\underline{en}^1$ heterozygotes (Table 8). In these flies, some portion of the posterior wing blades were transformed to haltere tissue, as shown in Figure 14. The area of transformation extended from the anterior-posterior boundary of the wing into the posterior compartment. The transformed area varied from a thin strip (Figure 14C) to a complete transformation of the posterior wing blade to haltere capitellum (Figure 14B). As with the previously described wing abnormalities, only distal structures were affected. As shown in Figure 14D, the transformed tissue contained haltere-like trichomes and sensilla trichodea. The abnormality never extended into the anterior compartment, although the small size of the haltere tissue sometimes distorted the shape of the anterior wing.

Table 8. Quantification of new abnormalities.

Genotype	Frequency of wing to haltere transformation[1]	Frequency of posterior claw abnormal or missing[2]
en1/en1	0 (5000)	0.004 (228)
en28/en1	0.004 (1364)	0.37 (746)
en28/en30	0.264 (4690)	0.86 (498)
en30/en1	0 (5000)	0 (210)
en28/+	0 (5000)	0 (210)

1. Number of wings examined shown in parentheses.
2. Number of legs examined shown in parentheses.
   Frequency shown represents pooled data for all three legs.

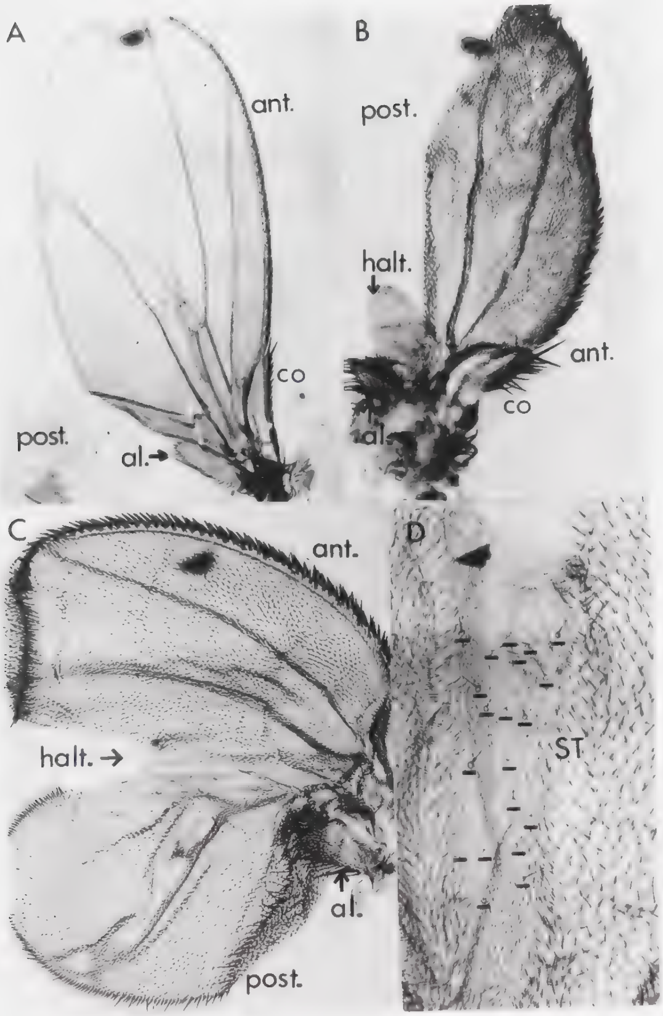


Figure 14. Abnormalities of en28/en30 wings, bright field optics.

A. magnification x20. Wing without transformation to haltere. Posterior compartment (post.) shows partial transformation to anterior (ant.).
B. x25. Entire posterior wing blade is transformed to haltere (halt.) tissue; anterior remains unaltered. Proximal posterior wing structures such as alula (al.) are still present.
C. x40. Strip of haltere tissue in wing blade is located just posterior to the position of the normal anterior-posterior boundary. Remainder of the posterior wing blade shows partial transformation to anterior. Note heavier bristles on alula, characteristic of normal anterior costa (co).
D. x100. Close up of wing shown in C, indicating sensilla trichodea (ST-underlined) which are characteristic of normal haltere tissue. Trichome size and density suggest haltere tissue rather than wing.

I considered the possibilities that this tissue was either part of the *engrailed* phenotype or was caused by a different locus. Several points mitigate against the second interpretation. First, as described above, extensive recombination of the backgrounds of *en28* and *en30* was undertaken. Recombinant *en28* chromosomes, which only carried the region of the original *en28* chromosome between *cn* and *vg*, still produced haltere tissue when heterozygous with *en30* or *en*^{1}. This suggests that a distant site on the second chromosome is not involved. In the recombination study described above (Figure 9), involving recombination between *en28* or *en30* and a multiply marked second chromosome, more than 5000 progeny were examined, each carrying one or both *engrailed* aberrations. Seven hundred and six flies showed *engrailed* abnormalities; of these, 357 also had one or both wings transformed to haltere. No flies were found which had the wing to haltere transformation, yet appeared to be otherwise *en*$^+$.

The wing to haltere transformation is seen not only in *en28*/*en30* flies, but also in *en28*/*en*^{1}, and *en30*/*enA* or *en30*/*enB* flies. These genotypic combinations have no single common allele, and come from completely independent genetic backgrounds, again suggesting that a locus other than *engrailed* is not required for the transformation.

Table 8 shows the frequency of abnormal claws and wing to haltere transformation in different genotypes. Frequency of claw abnormality varied among legs, with the third legs being abnormal somewhat more frequently than the second or first. Frequencies shown represent pooled data for all three legs. The genotypes can be ranked in a consistent order according to the frequency with which they show these new abnormalities (Table 7, Group 2). However, it is interesting to note that the ranking differs from that calculated for the PTR and PSCT abnormalities (Table 7, Group 1A). The new abnormalities (Group 2) are expressed most strongly in genotypes carrying a deletion of the *engrailed* locus. The Group 1A abnormalities are never expressed more strongly than in *en*^{1} homozygotes.

Since posterior wing vein and wing shape abnormalities did not follow the same order of expression as PTR and PSCT, I compared their expression to the abnormalities in Group 2. *en28*/*en30* showed significantly stronger expression than *en30*/*en*^{1}, which is compatible with the order found for Group 2. However, *en*^{1}/*en*^{1} still produced the highest mean expression for Group 1B, which is incompatible with the Group 2 ordering. An explanation for this observation in the posterior vein abnormality could rest with the method of classifying degree of expression. Presence of sensilla campaniformia on the transformed fourth vein was considered the highest degree of expression (see Figure 12). This placement of anterior sensory organs in the posterior compartment is similar to the production of PTR and PSCT. Including it as part of the vein abnormality may have skewed the results towards high ranking of expression in *en*^{1}/*en*^{1}. It can be concluded that while PTR and PSCT abnormalities are *en*^{1} specific and Group 2 abnormalities are most extreme when *engrailed* is deleted, alterations in posterior wing shape and vein pattern may result from more than one type of lesion at the *engrailed* locus.

It should be noted that the wing to haltere transformation is the one abnormality which is not affected in exactly the same manner when a genotype carries either *en28*, or *enA* or *enB*. The transformation is seen at low frequency in *en28*/*en*^{1}, but has not been observed in *enA*/*en*^{1} or *enB*/*en*^{1}. However the transformation is very rare even in *en28*/*en*^{1}, and may be further reduced by altering genetic background. The total number of *enA*/*en*^{1} and *enB*/*en*^{1} flies examined thus far is only about 300, so it is possible that the transformation will yet be observed.

Selection for increased expression of *engrailed*

Considerable variation among strains of a single genotype was observed in the expressivity and penetrance of all abnormalities discussed above, indicating that background modifiers influence the expression of *engrailed*. I wished to determine whether background modifiers might reveal elements of the *engrailed* transformation not previously observed. This question might be approached by outcrossing an *engrailed* stock to maximize the number of potential modifiers, then selecting and mating those flies which showed strong expression of the desired characteristics. This approach also offered the advantage of clarifying the relationship between different sets of abnormalities. If flies were selected on the basis of strong wing abnormality, I could ask whether leg abnormalities also increased; if selected for Group 1 abnormalities, I could determine the effect on Group 2 abnormalities.

Most flies of any *engrailed* genotype are sterile, making direct selection impossible. However, one homozygous *en*^{1} fertile stock was available, In(2L)Cy, *Cy cn en*^{1}/*lt stw en*^{1}. The In(2L)Cy, *Cy cn en*^{1} chromosome (hereafter referred to as *Cy en*^{1}) can be recovered over any other chromosome carrying *en*^{1}, and produces fertile adults. It cannot be recovered in homozygous state, due to the recessive lethality of the *Cy* mutation. This *Cy en*^{1} chromosome was thus chosen to be recovered over other *en*^{1} chromosomes so that *en*^{1} homozygotes could be selected and mated.

Figure 15 shows the series of crosses designed to randomize the genetic backgrounds of *en*^{1}/*en*^{1}, then select for strong expression of *engrailed* transformations. Selection was made solely on the basis of wing blade appearence; those flies in the F_2 generation which had the most dramatic alterations in wing shape and posterior vein pattern (i.e. Group 1B) were isolated and crossed among themselves. This procedure was repeated with their progeny (F_3). The progeny of this generation (F_4) were then examined without selection to determine if an overall increase in expression of

P 1 w;Gla/Cyo x w;lt stw en^1/Cy en^1

↙

F 1 w;Gla/Cy en^1 x cn en^1/SM5

↓

F 2 (w);cn en^1/Cy en^1 select and mate

F 3 (w);cn en^1/Cy en^1 select and mate

F 4 (w);cn en^1/Cy en^1 exmanine

Figure 15. Selection for strong expression of Group 1 abnormalities.

Cy en^1 chromosome indicates In(2L)Cy, *Cy cn en*^{1}. Selection was on basis of wing blade shape and posterior vein abnormalities. F4 flies were examined for other Group 1 and Group 2 transformations. See text for details.

Group 1 abnormalities had occurred.

Although the selection itself had been only on basis of wing shape and vein pattern, the progeny of the selected flies also showed an increase in Group 1A abnormalities. Frequency of posterior triple row bristles in the wings and posterior sex comb teeth in the legs had increased when compared with the original lt stw en^1/Cy en^1 strain (Table 9). However, the normal bristles and teeth of the anterior compartment also increased in frequency, making it difficult to determine whether modifiers were specific for engrailed characteristics or for a general increase in bristle number.

Close examination of the proximal posterior wing blade revealed a new element of transformation; many of the wings showed partial fusion of the alula to the wing blade (Figure 16). The only effect which has previously been reported for the alula of en^1/en^1 flies is the appearence of large socketed bristles resembling those which usually occur on the anterior proximal costa (Lawrence and Morata, 1976). These socketed bristles also appeared on the alulae showing fusion (Figure 16).

Penetrance of the alula fusion was incomplete in F_4 flies which had been grown at 22° C (Figure 16A). Among a random sample of 30 F_4 flies, 19 showed some degree of fusion of the alula of both wings. Six flies had one normal alula and five had both alulae unaffected, giving a penetrance of 73% for the alula abnormality. There is some tendency for any one fly to have neither or both wings affected; random distribution of the effect would predict 16 flies with both alulae, 12 with one, and two with neither alulae altered (Appendix 5, $X^2 = 7.08$, $p<0.001$).

Genetic background was not the only factor which could affect the expression of Group 1 abnormalities; it could also be increased by lowering the growth temperature (see below, under "Temperature sensitivity of engrailed effects"). In F_4 flies which had been grown at 18°, penetrance of the alula fusion approached 100%. The number of socketed bristles on the alula increased as well (Figure 16B,C). At this temperature,

Table 9. Increase in Group 1A abnormalities following selection.

Genotype	# Triple row bristles[1,2]		# Sex comb teeth[1,3]	
	Anterior	Posterior	Anterior	Posterior
Original strain $lt\ stw\ en^1/Cy\ en^1$	74.4±6.7 (22)	31.2±8.2 (22)	10.8±0.8 (24)	7.9±1.8 (24)
Progeny of selected strain $cn\ en^1/Cy\ en^1$	79.2±7.6 (60)	41.9±9.0 (60)	11.1±0.9 (14)	10.0±1.5 (14)

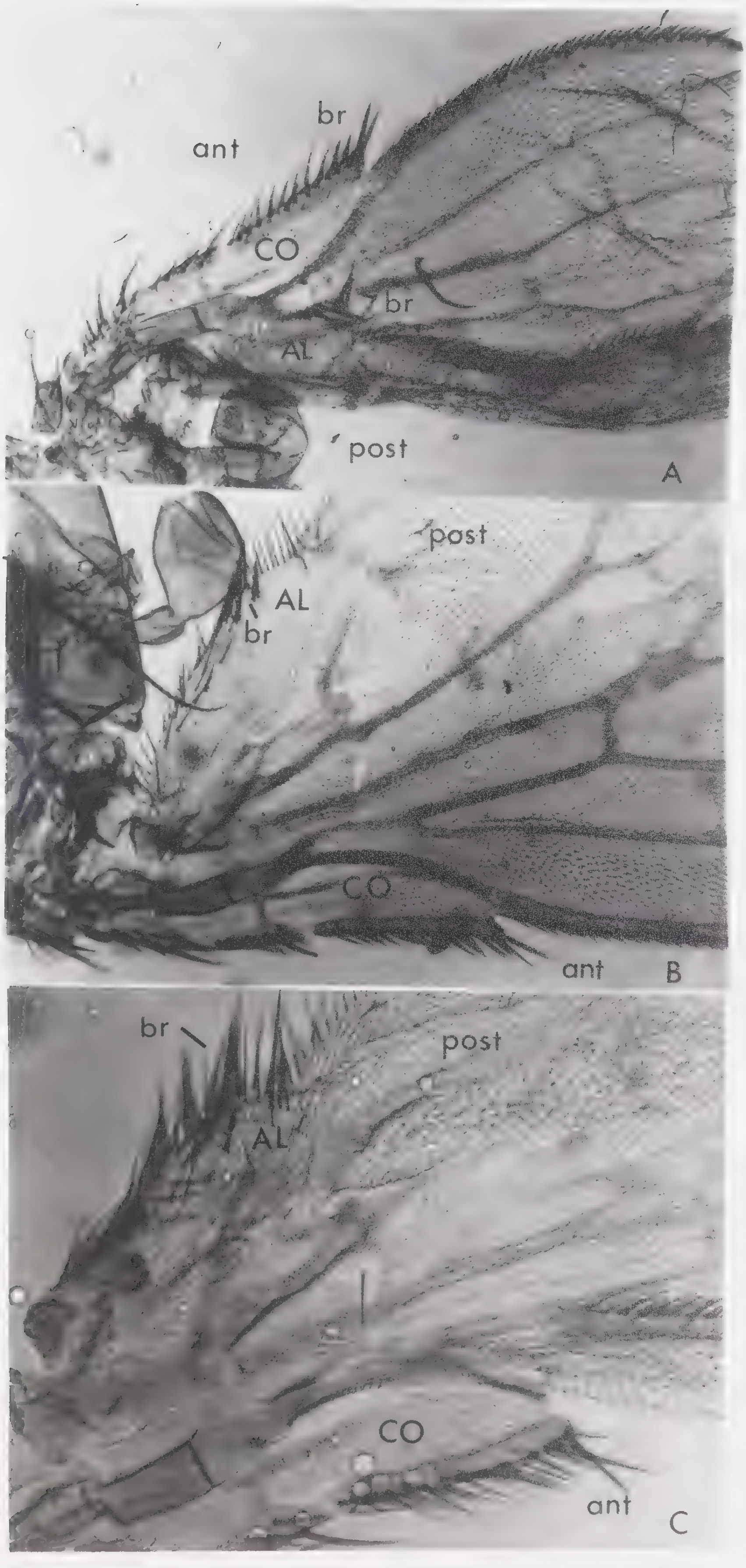
1. Mean ± standard deviation.
2. Number of wings examined shown in parentheses.
3. Number of legs examined shown in parentheses.

Figure 16. Alula abnormalities.

A. $\underline{en}^{1}/\underline{Cy}\ \underline{en}^{1}$ F_4 female grown at 22° C. Note incomplete separation of alula (AL) from posterior (post) wing blade. Bristles (br) characteristic of the anterior costa (CO) have appeared on the alula. magnification x60

B. $\underline{en}^{1}/\underline{Cy}\ \underline{en}^{1}$ female grown at 18°. Fusion of alula to wing blade is more complete, and more large socketed bristles have appeared on the alula. x100

C. $\underline{en}^{1}/\underline{Cy}\ \underline{en}^{1}$ male grown at 18°. number of socketed bristles on the alula has increased to nearly equal that on the costa. x100


br
ant
CO
br
AL
post
A
post
AL
br
CO
ant
B
br
post
AL
CO
ant
C

other group 1 characteristics showed very high expressivity. Frequently more sex comb teeth appeared in the posterior compartment of the male fore leg than in the anterior; one fly was found with 17 sex comb teeth in the posterior and only 11 in the anterior of the same leg. Many wings showed 60 or more posterior triple row bristles.

It is noteworthy that the increase in Group 1 abnormalities observed did not necessarily increase the resemblence of the transformed posterior compartment to normal anterior. Extreme increase in sex comb tooth number resulted in the appearence of more than one posterior comb row (Figure 17A) or of clumps of posterior teeth (Figure 17B). Extra medial triple row bristles on the posterior wing and socketed bristles on the alula were sometimes scattered in additional rows, rather than lining the wing margin in a single row as in the anterior.

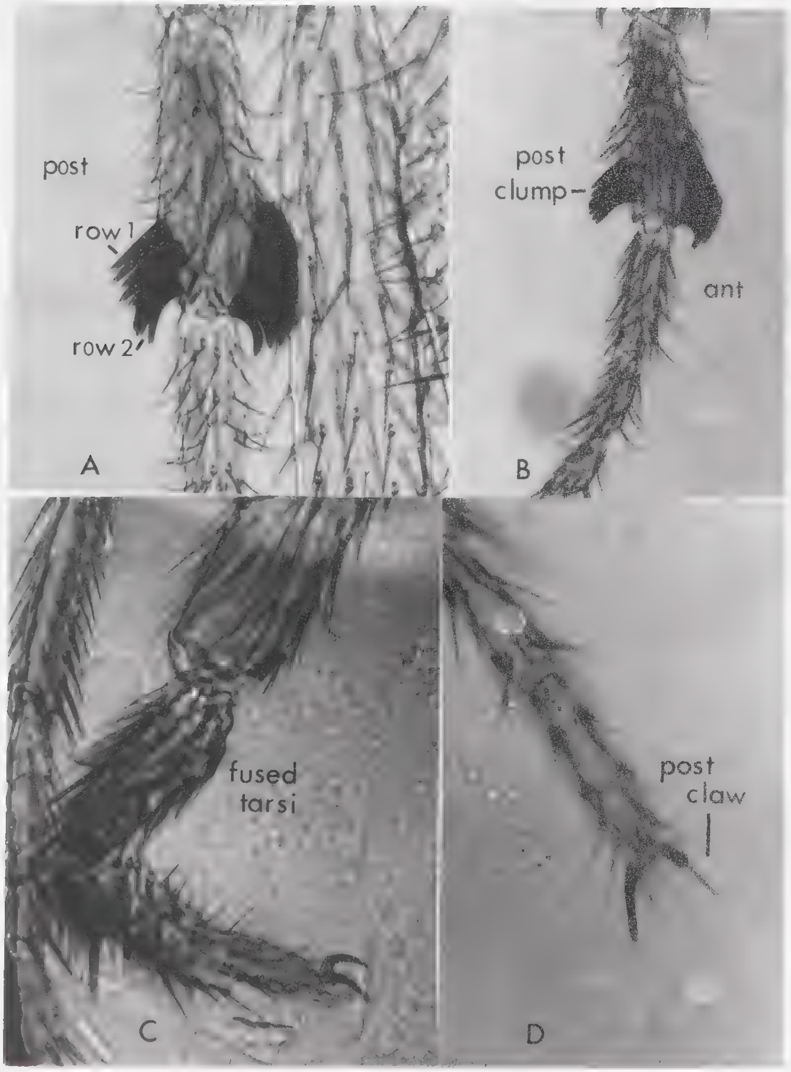
Following the selection procedure, F_4 flies were also examined for expression of Group 2 abnormalities. Although no flies showed transformation of the wing to haltere, several showed Group 2 leg abnormalities, including tarsal fusion (Figure 17C) and degenerate posterior claws (Figure 17D). A random group of 32 F_4 flies (grown at 22°) were mounted for close examination of the legs. Of 192 legs, 11 (5.7%) showed absence or degeneration of the posterior claw. In addition, 4 legs (2.1%) showed tarsal fusion. These abnormalities had not been observed in the legs of the parental strain. This suggests that selection for increased expression of Group 1B characteristics can result in simultaneous acquistion not only of other Group 1, but also of Group 2 abnormalities.

Relationship between Group 1 and Group 2 abnormalities

The data shown in Tables 5, 6, and 8 suggest a tendency for a genotype to express either Group 1A or Group 2 abnormalities strongly, but not both. Thus the possiblity existed that these groups of abnormalities might represent two mutually

Figure 17. Leg abnormalities in selected strains.

en^1/*Cy* *en*^{1} males show high numbers of posterior sex comb teeth (PSCT) arranged in more than one row (A.) or in clumps (B.) rather than in a single organized comb as in the anterior. These flies also show fused tarsal segments (C.) and degeneration of posterior claws (D.). All were grown at 22 . ant: anterior post:posterior
magnifications: x150, x100, x100, x200


post
row 1
row 2
A
post
clump
ant
B
fused
tarsi
C
post
claw
D

exclusive pathways of development. However, the above observation that both Group 1A and Group 2 characteristics may be acquired when selecting for Group 1B would suggest a correlation among the groups. I wished to determine:

1. whether Group 1A and Group 2 abnormalities could co-exist in the same structure (wing or leg)
2. whether, within a given genotype, there was any correlation between the expression of the two groups of abnormalities.

Male first legs were examined for presence of PSCT (Group 1A) and absence of degeneration of posterior claw (Group 2). Wings were examined for presence of PTR bristles (Group 1A) and transformation of posterior wing tissue to haltere (Group 2).

Table 10 shows the results of examining the two types of leg abnormalities in $\underline{en28}/\underline{en}^1$ and $\underline{enB}/\underline{en}^1$ males. A number of the legs examined showed both types of abnormalities (Figure 18A). The mean number of posterior sex comb teeth and the penetrance (i.e. number of legs with at least one posterior tooth) were compared between the legs in which both claws were normal and those with an abnormal posterior claw. For both genotypes, these numbers were very similar, indicating that the tendency for one type of transformation to occur is independent of the presence of the other transformation. A group of 67 $\underline{en28}/\underline{en30}$ legs were examined for this study as well, but none of them contained posterior sex comb teeth, making it impossible to draw a conclusion.

Examination of wings showed that both posterior triple row bristles and haltere tissue could occur in the same wing (Figure 18B). Indeed, transformation of the entire posterior wing blade to haltere could occur simultaneously with acquisition of long socketed bristles on the alula (see Figure 14B). Gathering data regarding the correlation between the two types of transformation proved difficult since, of the genotypes which show wing to haltere transformation, $\underline{en30}/\underline{en28}$ very seldom produces

Table 10. Correlation of Group 1 and Group 2 abnormalities in legs.

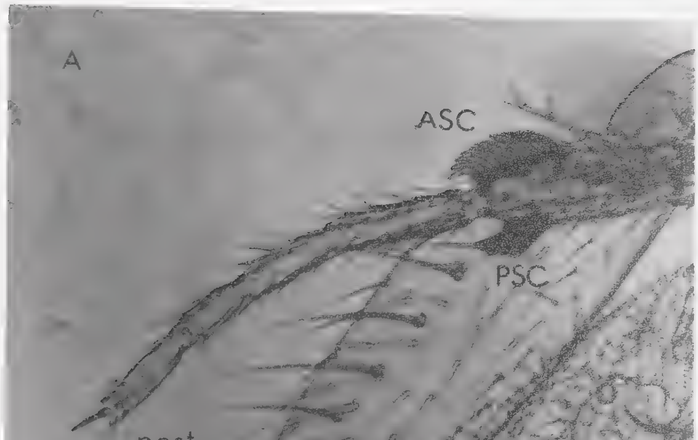
	# legs	# PSCT[1]	Mean # PSCT/leg[3]	#legs with 1 or more PSCT	Penetrance[2]
en28/en^{1}					
All legs	48	127	2.6±2.0	43	90%
Legs with 2 normal claws	38	99	2.6±1.8	34	89%
Legs with abnormal posterior claw	10	28	2.8±2.7	9	90%
enB/en^{1}					
All legs	44	214	4.9±1.8	44	100%
Legs with 2 normal claws	39	192	4.9±3.4	39	100%
Legs with abnormal posterior claw	5	22	4.4±1.5	5	100%

1. PSCT: posterior sex comb teeth.
2. Pentetrance = number of legs with one or more posterior sex comb teeth/total legs.
3. Mean ± standard deviation.

Figure 18. Coexistance of Group 1 and Group 2 abnormalities.

A. *en*^{1}/*en*^{1} male first leg showing both a posterior sex comb (PSC) in mirror image symmetry to the normal anterior sex comb (ASC), and a degenerate posterior (post) claw. magnification x 110.

B. *en28*/*en*^{1} wing showing both transformation of tissue to haltere (HALT) and production of triple row bristles along the posterior margin (PTR). x50


A
ASC
PSC
post claw
B
HALT
PTR

posterior sex comb teeth and *en28*/*en*^{1} rarely produces the transformation.

Two types of comparisons were made. Table 11 shows the results of scoring two different strains of *en28*/*en30* for both posterior triple row and wing to haltere transformation. As with the legs in Table 10, mean number of posterior triple row bristles and penetrance were compared between the wings with and without haltere tissue. Both expressivity and penetrance were slightly lower in those flies containing haltere tissue. This may be due to the fact that transformation to haltere reduces the size of the posterior margin, thereby leaving less area for triple row bristles to be produced. Alternately, it could indicate a real tendency for any given wing to show only one of the two possible transformation types. With regard to the second alternative, it is noteworthy that the first strain in Table 11 showed low expression and penetrance of the posterior triple row, but had a high frequency of transformation to haltere (25% of the wings). The reverse was true of the second strain, which had only 7% of the wings transformed to haltere, but higher PTR expression.

The second method of comparing expression of PTR and wing to haltere transformation was to examine *en28*/*en*^{1} flies which showed wing to haltere transformation in one wing only. Ten such flies were examined (Table 12). Once again, the mean number of PTR bristles and the penetrance were higher in the untransformed wings. In addition, eight of the ten flies exhibited the higher number of bristles in the untransformed wing.

Although a given structure could show both posterior to anterior and Group 2 type transformations simultaneously, the question remained as to whether a single cell could express both types of abnormality. To approach this question, I examined wings which showed transformation to haltere to determine whether the transformed tissue represented anterior or posterior haltere.

Table 11. Correlation between Group 1 and 2 abnormalities in en28/en30 wings.[1]

	# wings	#PTR	Mean #[2] PTR/wing	# wings with 1 or more PTR	Penetrance[3]
Sample 1					
Total wings	158	15	0.10±0.42	9	6%
Untransformed wings	118	12	0.10±0.44	7	6%
Wings transformed to haltere	40	3	0.08±0.35	2	5%
Sample 2					
Total wings	160	132	0.83±2.03	38	24%
Untransformed wings	149	127	0.85±2.07	36	28%
Wings transformed to haltere	11	5	0.45±1.21	2	18%

1. Group 1 abnormalities are represented by the number of posterior triple row bristles(PTR); Group 2 by transformation of wing tissue to haltere.
2. Mean ± standard deviation.
3. Penetrance = percentage of wings with at least one PTR bristle.

Table 10. Comparison of Group 1 and Group 2 expression in wings within one *en28*/*en*^{1} fly.[1]

	Normal wing	Transformed wing
# PTR bristles	24	23
	3	11
	6	3
	1	15
	12	1
	18	11
	18	1
	20	15
	7	2
	3	0
Mean # bristles[2]	11.2 ± 8.3	8.2 ± 7.9
Penetrance[3]	100%	90%
# flies in which this wing has higher # PTR	8	2

---

[1] Each pair of numbers represents the two wings of a single fly. Group 1 is represented by presence of posterior triple row (PTR) bristles; Group 2 by transformation of wing tissue to haltere.

[2] Mean ± standard deviation.

[3] Penetrance = percentage of flies showing at least one PTR bristle.

Simple examination of the tissue proved only partially enlightening because the only landmarks distinguishing anterior from posterior haltere in the capitellum are the sensilla trichodea (Hayes, 1982). Figure 19 is a diagram which shows the position of the sensilla trichodea (ST) on the normal haltere, as well as the anterior-posterior boundary as determined by clonal analysis (adapted from Hayes, 1982). There are two separated groups of ST on the capitellum, a proximal clump on the dorsal surface, which generally comprises from 3 to 8 sensilla, and an arc of 10 or more ST extending distally on the ventral surface. Both these groups show a degree of indeterminacy of location with respect to the A-P compartment boundary. The dorsal clump is usually in the posterior, although it sometimes straddles the boundary. The ventral group generally begins just anterior to the boundary, then curves anteriorly as it extends distally. Occasionally one or two of the more proximal ventral sensilla are found in the posterior compartment (Hayes, 1982).

If the haltere tissue found in engrailed wing blades is posterior haltere, proximal dorsal ST would be expected to be produced more frequently than the more distal ST on the ventral surface. If the haltere tissue corresponds to anterior haltere, then the reverse would be expected. Table 13 shows the results of examining wing to haltere transformed tissue in en28/en30, en28/en^1 and en30/enB flies. The haltere tissue in the engrailed wings contained at least as many ST as both compartments of the normal haltere, distributed in much the same manner between dorsal and ventral groups. This result did not clearly indicate compartmental identity.

In the second approach to the question of compartmental identity of the haltere-like tissue, I employed interactions of the abnormality with alleles of the Bithorax-Complex. This complex is involved in compartment specific transformation between wing and haltere (for review see Lewis, 1978). Homozygous bithorax (bx) flies have anterior haltere transformed to anterior wing; postbithorax (pbx) homozygotes have

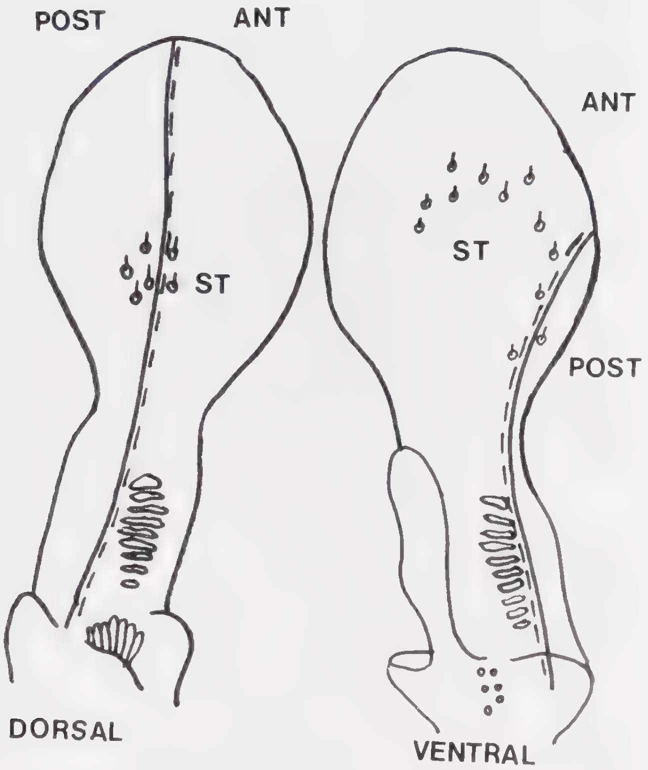


Figure 19. Diagram of normal metathoracic haltere (adapted from Hayes, 1982). Solid and dashed lines represent compartment boundary as determined by clonal analysis (Hayes, 1982). ANT: anterior compartment POST: posterior compartment. Typical distribution of sensilla trichodea (ST) in dorsal and ventral groups is shown.

Table 13. Number and distribution of sensilla trichodea in wing tissue transformed to haltere.

Genotype	Dorsal Group[1]		Ventral Group[2]	
	Range	Mean[3]	Range	Mean
en28/en30	0-8	4.0±2.8(26)	10-26	17.6±4.9(26)
en28/en^1	0-18	7.1±5.7(15)	0-17	9.2±4.5(13)
en30/enB	0-13	3.7±3.0(18)	5-32	17.5±7.4(13)

1. Number of wings examined is shown in parentheses.
2. Although both dorsal and ventral wing surfaces were examined in each wing, total number of dorsal and ventral groups recorded may differ due to transformation of only one surface in some wings.
3. Mean ± standard deviation.

posterior haltere transformed to posterior wing. This has been interpreted as meaning that bx^+ is required to make anterior haltere and pbx^+ to make posterior haltere. If the transformed tissue in en28/en30 wings is actually posterior haltere, it should require the pbx^+ gene. Table 14 shows that this is not the case; en28/en30;pbx/pbx flies show no reduction in the frequency of transformation of posterior wing to haltere when compared to their en28/en30;pbx/+ sibs. However, Table 15 shows that the bx^+ gene is required for this transformation to occur. This suggests that the tissue produced in the posterior compartment of the wing is anterior haltere, Thus the posterior to anterior transformation is also occurring in the mesothoracic cells which are transformed to haltere.

As noted above, the average number of ST in the transformed wing tissue was often greater than the average total in both compartments of the normal haltere. A possible explanation for the large number of ST is that haltere tissue undergoes greater than normal growth when surrounded by wing tissue. A similar observation was made when examining the ST pattern in wings transformed to haltere by the Polycomb mutation (Duncan and Lewis, 1982). This transformation differs from that caused bv engrailed in that the haltere tissue originates at the posterior margin and extends anteriorly; in extreme cases, tissue from both the anterior and posterior compartments may be affected. However, like the engrailed transformation, Polycomb produces high numbers of ST, even in weakly transformed wings. Duncan and Lewis note that partially transformed wings often contain more than 30 ST, although they do not indicate the dorsal or ventral location of the sensilla.

Since transformation of wing tissue to haltere could result in excessive numbers of ST, I wished to determine if simple juxtaposition of wing and haltere tissue could cause this phenomenon. Alternatively, an additional effect on the haltere-like tissue may be required. To test this, I examined the metathoracic halteres of bx^3/bx^3 flies.

Table 14. Incidence of wing to haltere transformation in en pbx flies.

	en28/en30;pbx/pbx	en28/en30 control[1]
# flies with both wings transformed	1	6
# flies with one wing transformed	5	45
# flies with neither wing transformed	16	114

Homogeneity $X^2 = 0.14$ $p > 0.90$

1. Control consists of all en28/en30 sibs which are not homozygous for pbx.

Table 15. Incidence of wing to haltere transformation in en bx flies.

	en28/en30;bx^3/bx^3	en28/en30 control[1]
# flies with both wings transformed	0	59
# flies with one wing transformed	0	159
# flies with neither wing transformed	21	248

Homogeneity $X^2 = 17.79$ $p < 0.005$

1. Control consists of all en28/en30 sibs which are not homozygous for bx^3.

In these flies, the anterior compartment of the haltere is transformed to wing, producing a metathoracic structure similar to the en28/en30 wing with posterior transformed to haltere.

When the en^+ allele was present, very few ST were present in either the dorsal or ventral groups in the posterior haltere (Table 16, bx^3/bx^3 sibs control). This result shows that mere presence of the faster growing wing tissue adjacent to the posterior haltere is not sufficient to cause the large number of ST seen in engrailed transformed wings. However, when any combinantion of mutant engrailed alleles was present in addition to bx^3/bx^3, an increase in the number of dorsal and ventral sensilla in the posterior haltere was noted (Table 16). Although this increase is not statistically significant when the variability of ST number is taken into account, the fact that there is always an increase suggests that engrailed may have an effect in the posterior haltere when bx^+ is not present.

Garcia-Bellido and coworkers (Garcia-Bellido, 1977; Garcia-Bellido, Lawrence and Morata, 1979) report an effect of en^1 on the haltere which they describe as a posterior to anterior transformation. However, they do not describe the criteria they use to distinguish anterior from posterior, nor show photographs of the alteration. I further examined the metathoracic halteres of all four engrailed genotypes in order to determine whether engrailed also altered ST pattern in the presence of bx^+.

Table 17 shows that the numbers of ST did not differ significantly between wild type flies and any of the engrailed genotypes tested. In addition, no trend towards increase in sensilla number was observed in the engrailed flies. This result is in agreement with that reported by Hayes (1982), but does not agree with the report of Garcia-Bellido et al. (1979). The alterations which they report may be dependent on background modifiers; this interpretation is possible in view of the observation that engrailed does affect ST number in combination with the presence of bx^3/bx^3.

Table 16. Effect of *engrailed* alleles on the sensilla trichodea pattern in the metathorax of *bx*^{3} flies.

engrailed genotype	*en**/*en**;*bx*^{3}/*bx*^{3} flies^{1,2} # sensilla trichodea			non-*engrailed*;*bx*^{3}/*bx*^{3} sibs^{1,2} # sensilla trichodea			Ratio of mean in *en**/*en** flies to sibs	
	dorsal		ventral	dorsal		ventral	dorsal	ventral
en^1/en^1	1.8 ± 1.5	(63)	4.5 ± 2.5	1.0 ± 1.4	(38)	2.2 ± 1.8	1.8	2.1
en28/en^1	3.2 ± 3.0	(34)	4.4 ± 2.8	1.4 ± 1.5	(17)	1.8 ± 1.9	2.3	2.5
en28/en30	2.2 ± 2.1	(15)	7.1 ± 3.4	1.3 ± 1.9	(9)	2.0 ± 1.6	1.7	3.5
en30/en^1	0.9 ± 1.4	(24)	6.3 ± 2.8	0.8 ± 1.2	(20)	3.8 ± 2.2	1.2	1.7

en* may refer to any of the three *engrailed* alleles.

1. Mean ± standard deviation.
2. Number of halteres examined shown in parentheses.

Table 17. Distribution of sensilla trichodea in the metathoracic haltere.[1]

Genotype	# halteres examined	Dorsal group[2]	Ventral group[2]
wild type	35	3.8 ± 2.2	12.0 ± 2.1
en^1/en^1	23	4.2 ± 1.5	13.2 ± 3.0
$en28/en^1$	30	4.7 ± 1.5	12.9 ± 2.6
en28/en30	34	3.6 ± 1.7	12.5 ± 2.3
$en30/en^1$	24	4.6 ± 1.5	11.7 ± 2.0

1. Normal distribution of sensilla trichodea is shown in Figure 19.
2. Mean number of sensilla in group ± standard deviation.

It is possible that the haltere pattern alteration observed in *en*;*bx*^{3} genotypes is an entirely non-specific effect caused, for example, by the longer development time required by *engrailed* flies. This interpretation is consistent with the observation that the increase in ST number is greatest in the genotypes with the slowest development (*en28*/*en30* and *en28*/*en*^{1}). Alternatively, the effect could be attributed to *engrailed*. Expressivity in the four genotypes is consistent with placement in Group 2 (i.e. *en28*/*en30* > *en28*/*en*^{1} > *en*^{1}/*en*^{1} > *en30*/*en*^{1}; see Table 7).

A final observation on the effects of *engrailed* in the haltere is that, even in combination with alleles of the Bithorax-Complex, only the posterior haltere is affected. This was ascertained by combining *engrailed* genotypes with *pbx*/*pbx*, and examining the sensilla pattern in the untransformed anterior haltere. As shown in Table 18, neither the presence of *en28*/*en30* nor of *en28*/*en*^{1} produced a significant difference in the ST pattern of *pbx*/*pbx* flies when compared to the *en*$^{+}$ sibs. In addition, no consistent trend can be observed. This is not surprising, since effects of *engrailed* have not previously been observed in anterior compartments of adults.

Table 18. Effect of engrailed on the sensilla trichodea pattern in the anterior metathorax of pbx/pbx flies.

Genotype	Number examined	Dorsal Group Range	Dorsal Group Mean[1]	Ventral Group Range	Ventral Group Mean
en+;pbx/pbx sibs	23	0-4	1.0±1.1	11-21	16.4±3.1
en28/en30;pbx/pbx	31	0-5	1.1±1.5	8-20	13.2±3.3
en28/en^1;pbx/pbx	7	0-2	0.4±0.8	12-20	15.9±2.8

1. Mean ± standard deviation.

Effects of *engrailed* in other segments

The analysis reported thus far has centered on the effects of *engrailed* in the thoracic segments. Several *engrailed* alleles have been reported to affect head, abdomen and genital segments as well (Morata and Lawrence, 1978; Kornberg, 1981b; Lawrence and Struhl, 1982; Epper, 1980).

Genital abnormalities of males carrying *en28* or *en30* were analysed by F. Epper (personal communication). He found the most extreme abnormalities in *en28*/*en30* males, where most of the genital structures were absent, leaving only the genital arch and lateral plate. This abnormality was very consistent, and showed complete penetrance. *en28*/*en*^{1} males were less strongly affected; in many cases, a reduced penile apparatus was formed in addition to the structures found in *en28*/*en30*. Males of *en30*/*en*^{1} genotype were even closer to normal in appearence, often forming claspers as well as a penile apparatus. Since Epper found little or no abnormality in the genitalia of *en*^{1}/*en*^{1} males (Epper, 1980), this abnormality falls into Group 2 with respect to expressivity and penetrance in different genotypes (i.e. *en28*/*en30* > *en28*/*en*^{1} > *en30*/*en*^{1} > *en*^{1}/*en*^{1}).

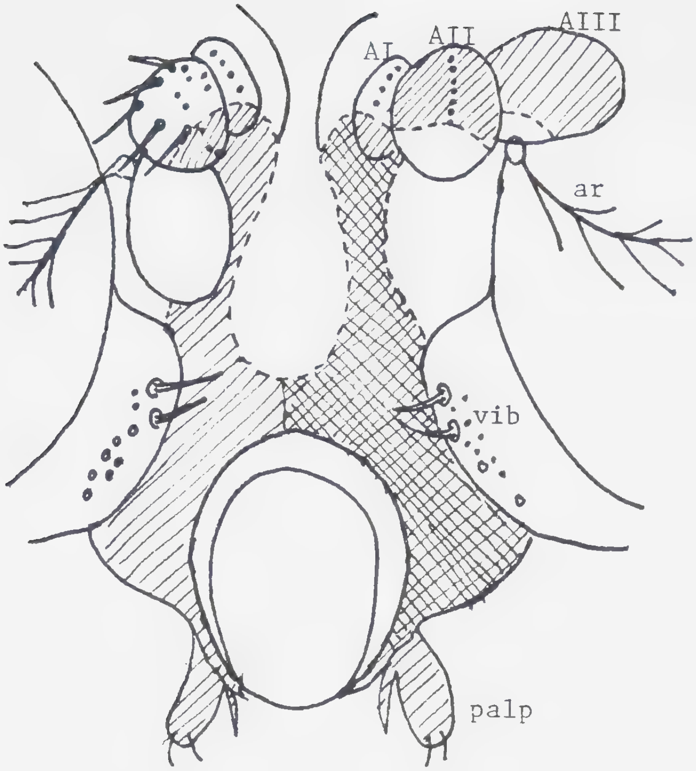
Engrailed has also been reported to act in the antenna (Morata and Lawrence, 1978, 1979). Antennal abnormalities have never been reported in *en*^{1} homozygotes, but Morata and Lawrence observed duplication of the second and third antennal segments and arista occurring with low penetrance in *en*^{1}/*en*^{C2} flies. They reported a consistent plane of symmetry about which the antennal structures duplicated, and contended that this plane represented the anterior posterior compartment boundary (Figure 20A). According to their hypothesis, the duplication of anterior structures was a replacement of the posterior compartment by anterior, corresponding to the posterior to anterior transformation in the wing.

I examined the head structures in several strains of each of the genotypes: *en*^{1}/*en*^{1}, *en28*/*en*^{1}, *en28*/*en30* and *en30*/*en*^{1}. Antennal alterations with some similari-

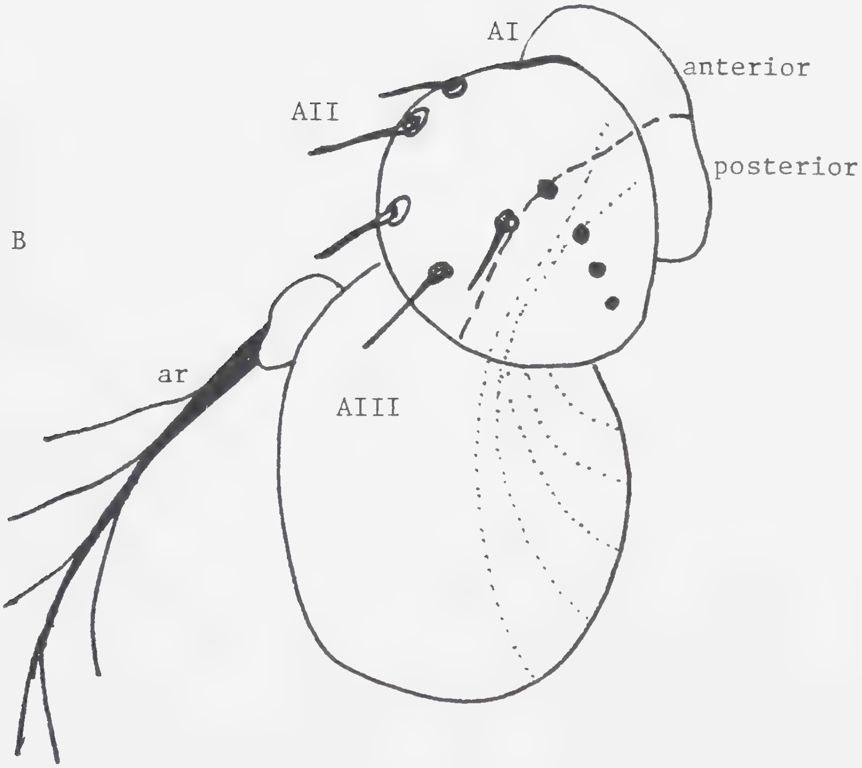
Figure 20. Antennal structures and compartments.

A. Antennal disc derivatives showing anterior-posterior compartment boundary, from Morata and Lawrence (1978). AI, AII, AIII: first, second and third antennal segments. ar: arista. palp: maxillary palp. vib: vibrissae. Large bristles are shown; solid circles represent small bristles. Dashed line shows anterior-posterior compartment boundary. Blank region is anterior compartment, hatched region is posterior compartment. Double hatched region of posterior compartment shows region referred to as "blank tissue" in Table 19. Note that the revised compartment map of Struhl (1981) indicates that the maxillary palp comprises both anterior and posterior tissue.

B. Diagram of three antennal segments and arista, dorsal view. Abbreviations as above. Dashed line shows anterior-posterior compartment boundary in AI and AII; portion of AIII shown, and arista, are entirely anterior. Dotted lines show planes of duplication observed in *en*^{1}/*en28* heads. These duplications rarely produce a second arista.



A



B

ties to those reported by Morata and Lawrence occurred at low frequency in a single *en*^{1}/*en28* strain. Other *engrailed* genotypes and other strains carrying the same *engrailed* alleles never showed these abnormalities.

The abnormalities observed in *en*^{1}/*en28* flies differed in several respects from those reported by Morata and Lawrence. First, there was no consistent plane of duplication in the antenna (Figure 20B), which could correspond to the anterior-posterior boundary as defined by Morata and Lawrence. Although they report duplication of the (anterior) arista in most cases, this was observed in only two *en*^{1}/*en28* heads, out of more than 300 abnormal heads examined. In two additional cases, structures were formed in the position expected for a duplicated arista. However, the duplicate structures were very abnormal and possibly represented transdetermination of a portion of the arista to leg (Figure 21A). However, bracted bristles were not discernible on the abnormal tissue, so it could not be definitely identified as leg.

If the head abnormalities were caused by transformation of the posterior compartment to anterior, several characteristics would be expected. First, only posterior structures should be reduced, absent, or abnormal. Second, only anterior structures should be duplicated or enlarged. Third, there should be a correspondence between the amount of posterior material missing and the amount of duplicated anterior.

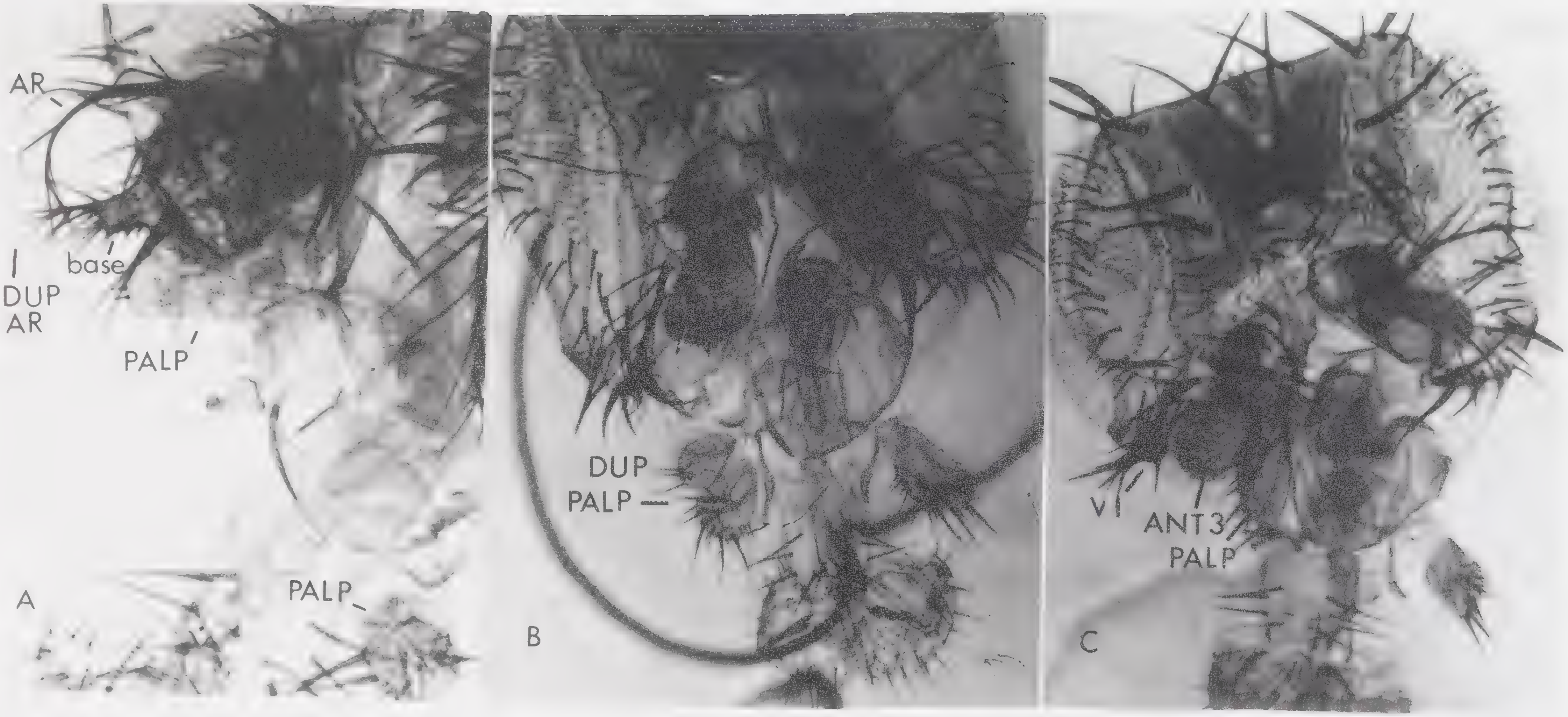
An alternative explanation for the head abnormalities is cell death and duplication. In this case, one might postulate an increased propensity towards death of cells in the posterior compartment; death of posterior cells could be followed by duplication of adjacent anterior cells, and produce structures that resembled posterior to anterior transformation. However, this mechanism could also produce abnormalities not predicted by transformation. Cell death without subsequent duplication would produce only abnormal or missing structures. Conversely, small areas of

Figure 21. Head abnormalities in en28/en^1.

A. Maxillary palp (PALP) is fused to the third antennal segment on left side while right palp is in normal position. In addition to the normal arista, there is an outgrowth from the antenna which resembles a duplicated arista distally (DUP AR), but which is very abnormal near the base. magnification x220

B. Maxillary palp is duplicated (DUP PALP) with very little reduction in the other structures. x130

C. Base of the third antennal segment (ANT3) and palp are fused, eliminating the intervening tissue and causing the vibrissae (VI) to form a clump. No structures appear markedly enlarged or duplicated. x110


AR
DUP
AR
base
PALP
PALP
A
DUP
PALP
B
V
ANT3
PALP
C

cells death might be followed by large amounts of duplication and cause an increase in the total number of structures present. Thus there need not be a close correspondence between areas of missing and duplicated tissues. In addition, posterior cell death may be followed by duplication of adjacent posterior cells, and result in duplicated posterior structures. It is noteworthy that head abnormalities caused by a temperature sensitive cell death mutation (Russell, 1974) resemble those observed in engrailed heads.

Table 19 shows the range of abnormalities observed in $\underline{en}^1$/$\underline{en28}$ heads. Structures considered have been divided into three groups, anterior only, mixed anterior and posterior, and posterior only. It should be noted here that the compartment maps of Morata and Lawrence (1978) place the maxillary palp entirely in the posterior compartment. However, the revised map reported by Struhl (1981) shows the palp to consist of both anterior and posterior tissue. I found the palp to be duplicated in many of the abnormal flies (e.g. Figure 21B). Were the palp entirely posterior, this observation would be inconsistent with the posterior to anterior transformation hypothesis. If the palp contains both anterior and posterior tissue, duplication of the palp does not, in itself, rule out the transformation hypothesis. For the present analysis, the palp is considered to comprise both anterior and posterior tissue.

While posterior structures were often absent in abnormal heads, neither missing nor duplicated structures were entirely posterior specific. Wholly anterior strutures (arista and vibrissae) were seldom or never duplicated. Vibrissae were often abnormal in that the usual lines of bristles were crowded together into a reduced space. This generally occurred in conjunction with reduced area of posterior "blank tissue" (see Figure 20A), and may have been a secondary effect. Most duplications were of structures containing both anterior and posterior tissue (antenna and palp),

Table 19. Type of abnormalities observed in en28/en[1] heads.

Type of abnormality	Temperature	# heads[1]	Anterior only		Anterior and Posterior			Posterior only
			Arista	Vibrissae[2]	AntII	AntIII	Palp[3]	Blank tissue[4]
Duplication or enlargement of structures	22° C	20	0	0	3	9	10	0
	25° C	15	1	0	7	16	5	0
	29° C	21	2	0	14	14	5	0
Absence, reduction, or abnormality of structures	22° C	20	0	12	0	0	6	13
	25° C	15	0	13	0	0	12	15
	29° C	21	1	20	0	1	14	22

1. Only abnormal heads are considered here, each head having two sets of structures. Thus the total number of structures recorded may exceeed the number of heads.
2. All vibrissa abnormalities were due to crowding of the rows of bristles into disorganized clumps. This generally occurred in conjunction with reduction of the area of blank tissue. Marked changes in bristle number were not observed.
3. For this table, the palp is considered to consist of both anterior and posterior tissue, as found by Struhl (1981), rather than posterior only as determined by Morata and Lawrence (1978).
4. For location of the posterior compartment "blank tissue", see Figure 20.

suggesting a possible role for the compartment boundary in duplication. However, neither antennae nor palps showed consistent mirror image duplication which defined the compartment boundary. In some cases the entire third antennal segment or palp was duplicated; thus duplication of posterior tissue could occur.

A strict correlation between loss and duplication of tissue was not observed. In some heads duplications were observed without any apparent reduction in other structures (Figure 21B). Alternately, absence or reduction of the posterior blank tissue separating the antenna and palp was a frequent abnormality. Simple elimination of this tissue sometimes occurred in the absence of any extensive duplication (Figure 21C).

Considered together, the above results suggest that posterior cell death, sometimes followed by duplication, is a more likely mechanism to produce *engrailed* head abnormalities than is redetermination of posterior cells to make anterior structures. One further test of this hypothesis considered the assumption that posterior cells in the heads of *en*^{1}/*en28* flies were unusually prone to cell death. If so, factors which increase frequency of cell death should show a disproportionately strong effect on these cells, and cause increased frequency of abnormality. Since X-irradiation causes cell death, *en*^{1}/*en28* larvae were iradiated (950r) at different times throughout development (Table 20). The frequency of head abnormalities was compared to that in unirradiated controls and in *en*/*en*$^{+}$ sibs irradiated at the same age. Table 20 shows that irradiation caused a marked increase in the head abnormalities in *en*^{1}/*en28* flies without affecting sibs. All head abnormalities were qualitatively similar to those observed in unirradiated *en*^{1}/*en28* flies. Thus cell death probably has a role in producing these abnormalities.

Table 20. Effects of X-irradiation on head abnormalities in en[1]/en28 flies.

Time of irradiation[1]	Total flies	# heads with one side abnormal	# heads with both sides abnormal	% abnormal half heads
Unirradiated control	296	3	0	0.5 %
en28/en[1] flies				
0-24 h AEL[2]	16	2	1	12.5 %
24-48 h AEL	9	0	1	5.6 %
48-72 h AEL	109	6	1	3.7 %
72-96 h AEL	78	13	1	9.6 %
> 90 h BPF[3]	191	38	22	21.5 %
66-90 h BPF	65	4	0	3.1 %
42-66 h BPF	50	2	0	2.0 %
later than 42 h BPF	20	0	0	< 2.5 %
en*/+ sib controls				
0-24 AEL	40	0	0	< 1.3 %
24-48 AEL	25	1	0	2.0 %
48-72 AEL	132	0	0	< 0.4 %
72-96 AEL	129	1	0	0.4 %
> 90 h BPF	231	0	0	< 0.2 %
66-90 h BPF	60	1	0	0.8 %
42-66 h BPF	45	0	0	< 1.1 %

1. All irradiations were 950r.
2. AEL: hours after egg laying.
3. BPF: hours before puparium formation.

Temperature sensitivity of engrailed effects

Brasted (1941) reported that the effect of en^1/en^1 as measured by the number of posterior sex comb teeth produced was cold sensitive. She found a steady increase in the mean number of posterior sex comb teeth produced as growth temperature was reduced, from 0.13 at 29.5°, to 6.26 at 14°. This increase was not correlated with an increase in the overall size of the fly as temperature was decreased. Lawrence and Morata (1976) reported a similar cold sensitivity in the wing transformation of en^1 homozygotes. They observed more extreme transformation at 25° than at 29° as quantified by counting the number of posterior triple row bristles present at each temperature. The clefted scutellum however, did not follow the same pattern of cold sensitivity. Brasted (1941) found that its expression was most extreme at 25° and decreased at more extreme temperatures.

These observations prompted several questions. I wished to determine whether the remainder of the abnormalities observed in en^1/en^1 flies were cold sensitive, and if these abnormalities also showed temperature sensivity in other genotypes. Of particular interest was the effect of temperature on the en28/en30 flies; if temperature sensitivity were an allele specific effect, it would not be observed here. In addition I wished to determine whether Group 2 abnormalities were affected by temperature.

Tables 21, 22, and 23 show the effect of temperature on Group 1 and Group 2 abnormalities. In order to avoid differences due to background modifiers, the same strains were examined at high and low temperature. Although differences are not always significant, the penetrance and expressivity of every abnormality examined were consistently higher at 22° than at 29°.

Flies raised at lower temperatures tend to grow larger than those raised at higher temperatures as measured by mean body weight (Neel, 1940). In addition, the

Table 21. Effects of temperature on expressivity of Group 1 abnormalities.[1,2]

	Temperature	en^1/en^1	$en28/en^1$	en28/en30	$en30/en^1$
posterior	22° C	41.5±8.1	9.8±7.1	0.7±2.0	0.3±0.7
triple row		(24)	(72)	(104)	(40)
bristles	29° C	23.1±11.7	2.9±4.8	0.1±0.6	0.1±0.7
		(40)	(110)	(84)	(48)
posterior	22° C	8.9±4.0	2.4±2.5	0.03±0.17	0.1±0.2
sex comb		(18)	(18)	(36)	(20)
teeth	29° C	5.5±4.4	1.4±3.2	0 (38)	0 (20)
		(21)	(26)		
posterior	22° C	4.8±0.4	4.4±0.5	4.0±0.5	2.3±0.6
wing veins		(26)	(34)	(50)	(40)
(scale 1 to 5)	29° C	3.5±1.1	3.5±0.7	3.0±0.7	1.5±0.6
		(31)	(38)	(44)	(48)

1. Mean ± standard deviation.
2. Number of wings or legs examined is shown in parentheses.

Table 22. Effect of temperature on penetrance of Group 1A abnormalities.[1]

Genotype	Temperature	# wings showing[2] PTR bristles	Penetrance	# legs showing[3] PSCT	Penetrance
en^1/en^1	22°	24 (24)	100 %	18 (18)	100 %
	29°	39 (40)	98 %	19 (21)	91 %
$en28/en^1$	22°	66 (72)	92 %	11 (18)	62 %
	29°	58 (110)	53 %	8 (26)	31 %
en28/en30	22°	19 (104)	18 %	1 (36)	3 %
	29°	5 (84)	6 %	0 (38)	< 3 %
$en30/en^1$	22°	7 (40)	18 %	1 (20)	5 %
	29°	2 (48)	4 %	0 (20)	< 5 %

1. Penetrance is percentage of structures showing transformation.
2. Number of wings examined shown in parentheses. PTR: posterior triple row bristles.
3. Number of legs examined shown in parentheses. PSCT: posterior sex comb teeth.

Table 23. Effect of temperature on the frequency of Group 2 abnormalities.

Abnormality[1]	Temperature	*en*^{1}/*en*^{1}	*en28*/*en*^{1}	*en28*/*en30*	*en30*/*en*^{1}
Wing to haltere transformation	22° C	0(2000)	0.013(720)	0.37(494)	0(2000)
	29° C	0 (500)	0.002(580)	0.26(254)	0 (500)
Posterior claw abnormal	22° C	0 (246)	0.366(745)	0.859(498)	0 (240)
	29° C	0 (162)	0.131(198)	0.675(708)	0 (192)

1. Number of wings of legs examined is shown in parentheses.

mean length of the wing itself increases at lower temperature (Stanley, 1935). Thus I had to consider the possibility that the increased numbers of posterior sex comb teeth and posterior triple row bristles were due to general size increase rather than a specific engrailed effect. Were this the case, the relative frequency of the normal anterior bristles would be much the same at the high and low temperatures as the relative frequency of the posterior engrailed structures. Table 24 shows the results of examining both anterior and posterior triple row bristles and sex comb teeth in wings and legs of two engrailed genotypes. Although the frequency of the anterior elements is decreased at 29°, the decrease is nowhere near as extreme as that observed for the engrailed transformed posterior elements. This indicates that the cold sensitivity is a real engrailed effect rather than an artefact.

One exception to the cold sensitivity rule was observed. Head abnormalities in en^1/en28 flies showed increased expression at higher temperatures. Not only was frequency of the abnormality increased (Table 25), but the type of abnormality changed as temperature was increased (Table 19). At low temperature, duplications of the palps and antenna were observed, seldom associated with missing structures. As temperature increased, more dramatic abnormalities appeared, with large areas of tissue absent, and frequent fusion of the palp to the base of the antenna. This may suggest larger areas of cell death occurring at higher temperatures. It does raise the question of how the antennal abnormalities are related to the other engrailed effects.

Two general conclusions can be drawn from the temperature sensitivity tests: cold sensitivity is not an allele specific effect, but is characteristic of several mutations of the engrailed locus, and cold sensitivity is a characteristic of the Group 2 abnormalities as well as Group 1.

Table 24. Effect of temperature on frequency of normal anterior elements compared to <u>engrailed</u> posterior elements.

		# wings examined	# triple row bristles[1]		# legs examined	# sex comb teeth[1]	
			anterior	posterior		anterior	posterior
en^1/en^1	22^o	14	75.8±6.7	36.3±4.9	18	11.1±1.1	8.9±4.0
	29^o	24	71.3±6.4	24.3±11.0	21	10.9±0.9	5.5±4.4
Ratio $29^o/22^o$			0.94	0.67		0.98	0.62
$en28/en^1$	22^o	14	82.4±6.4	10.1±6.8	18	11.9±1.2	2.4±2.5
	29^o	14	68.4±6.2	2.1±4.5	26	10.3±1.0	1.4±3.2
Ratio $29^o/22^o$			0.83	0.21		0.87	0.58

[1] Number shown is mean ± standard deviation.

Table 25. Effects of temperature on the frequency of en28/en^1 head abnormalities.

Temperature	# Heads examined[1]	# Abnormal	% Abnormal
22° C	518	9	1.7 %
25° C	246	11	4.5 %
29° C	102	7	6.9 %

1. All heads of en28/en^1 flies were mounted between coverslips and examined for abnormalities. Each head consists of two sets of structures, i.e. half heads. Abnormalities may affect one or both halves; frequency shown is on a per head, not a per half head basis.

DISCUSSION

Dosage relationships of *engrailed* alleles

A commonly applied method of learning about the wild type function of a gene is to examine the effects of mutations in that gene. For this to be effective, however, the nature of the mutations must be understood. If a mutation which alters the gene product is interpreted as though it eliminates the gene product, the conclusions reached regarding the normal function of the gene will be erroneous.

The first question to be approached in this regard is whether *en*^{1} is a hypomorph. This hypothesis has been advanced to explain why *en*^{1}/*en*^{1} causes only an incomplete posterior to anterior transformation of the wing, and assumes that absence of *engrailed* activity would produce a complete transformation. This assumption is critical for the hypothesis that *engrailed* is a selector gene which suppresses the anterior pathway of development, and promotes posterior. The hypothesis that *en*^{1} is a hypomorph has been tested by performing dosage studies with *en*^{1} and the deficiencies *en28* and *en30*.

It is unlikely that both *en28* and *en30* entirely delete the *engrailed* locus because they produce distinctly different phenotypes. Since *en30*/*en*-lethal flies survive, it can be concluded that *en30* does not remove the entire locus. However, *en28* removes most of 48A, where *engrailed* has been localized, and produces the same phenotype in all genotypic combinations as do the larger deficiencies *enA* and *enB*. This suggests that *en28* is entirely deficient for the *engrailed* locus. An additional point favoring this conclusion is that *en28* was isolated with the insertion on 3L which suppresses all *engrailed* effects; this probably occurred by transposition of the entire *engrailed* gene. Preliminary results of the molecular analysis of *engrailed* also indicate that the locus has been completely deleted in *en28* (T. Kornberg, personal communication).

Given that *en28* completely deletes *engrailed*, any abnormality which is weakly expressed in en^1/en^1 because of residual en^+ activity should be more strongly expressed in *en28*/en^1. This ranking of genotypes was obtained for abnormalities of the posterior compartment of legs, such as missing claws, and for the wing to haltere transformation (Table 7, Group 2). However, the effects of *engrailed* which can be unambiguously described as posterior to anterior transformations are expressed most strongly in the presence of two copies of the en^1 allele and more weakly when the *engrailed* locus is deleted (Table 7, Group 1A). This suggests that the posterior to anterior transformations are not due simply to a lack of en^+ activity. Possibly they arise from an altered or new activity conferred by the en^1 allele. This hypothesis is somewhat tenuous, since a mutation which causes alterations by producing a new or altered gene activity might be expected to be dominant over its wild type allele. However, en^1 might interfere with a normal process in a dosage dependent manner; en^1/en^+ would have sufficient en^+ activity to appear normal whereas en^1/en^1 would not, and the posterior to anterior effects produced by the en^1 allele would be weaker in en^1/deficiency genotypes with only one dose of en^1 present. At least one case has been recorded where a mutant acts in a manner similar to this, as a recessive antimorph (Thierry-Mieg, 1982).

Although the dosage studies indicate that en^1 is not a simple hypomorph, there is a drawback to this type of analysis. There may be regulative interactions between the two alleles of a gene, and regulation may be different when two gene copies are present than when one copy is deleted. For example, transcription of *engrailed* may require a threshold level of inducer. Local concentration of inducer may be higher when only one *engrailed* gene is present, the other deleted, resulting in overproduction of the allele which is present. If such a situation exists, it could result in en^1/*en28* producing more gene product and showing a more normal phenotype

than en^1/en^1. Until the nature of the regulation of engrailed is known, such a possibility cannot be eliminated.

A final alternative is that en^1 causes over-production of the normal engrailed gene product. This hypothesis can be ruled out by the observation that the insertion portion of the en28 transposition (i.e. the duplication of the $engrailed^+$ locus) suppresses all the abnormalities conferred by en^1. In addition, this insertion is homozygous viable and phenotypically normal in wild type background, indicating that four copies of the $engrailed^+$ gene will not alter phenotype.

Like en^1, the en30 deficiency does not have a readily defined effect on the engrailed locus. The observation that en30/en28 and en30/en-lethal flies survive shows that en30 does not inactivate the engrailed vital locus. However, since both these genotypes show adult abnormalities, there is some effect on the engrailed gene, possibly a position effect.

Number of gene functions

All the above considerations are built on the assumption that the engrailed phenotype is the result of a mutation involving only one gene function. It is possible that engrailed is a complex locus with at least two different (although perhaps related) functions. The genetic evidence which would support such a hypothesis is that two types of allele dependent abnormalities are observed. Lethal alleles form one complementation group which produce extreme embryonic abnormalities. Both en^1 and en30 complement the lethals with respect to the embryonic phenotype, but produce adult abnormalities. However, the adult phenotype of the lethal alleles over en^1 or en30 is very mild compared to these alleles over en28 or to en^1 homozygotes. These observations may indicate the existence of one gene function required for proper embryonic development, and a second gene function required only in the adult. Two gene functions could be ascribed to two separate genes or to a single structural

locus, the product of which is differentially processed and thus functions differently early and late in development.

If separate embryonic and adult functions are involved, en^1 affects only the adult function, en-lethals only the embryonic function, en28 deletes both, and en30 has some effect on the adult function, but does not inactivate the embryonic one. However, the two functions are not completely independent, since en^1/en-lethal adults show some abnormality. In addition, clones of lethal alleles are abnormal in many segments, even when induced late in development. Thus the early gene function continues to be required after embryogenesis. This could indicate a dependence on the presence of the normal embryonic gene (in cis) for proper regulation of the adult gene. For example, if alternate processing of a single transcript is involved, en-lethal mutations might abolish the early function and alter processing so that the late gene product is not completely normal.

The observed genetic relationship might also result from mutation of genes for two steps in the same biochemical pathway required for normal adult differentiation. If each step is sensitive to the level of normal gene product, mutations in the genes for the two steps could fail to complement completely, giving mild adult abnormalities. The normal appearence of (for example) an en^1/en-lethal embryo could indicate that either 1) lower levels of the pathway end product are required in the embryo or 2) only the en-lethal gene product is required in a different pathway in the embryo.

Biochemical evidence in support of the two gene hypothesis is Kornberg's report of two RNA's transcribed from the engrailed region (personal communication). It is not yet clear how these transcripts are related, and whether there are spatial or temporal differences in their expression.

Evidence against the existence of two separable gene functions derives from the results of mutagenesis screens. Even when screens are designed to identify alleles

causing adult abnormalities over *en*^{1}, the induced alleles tend to be embryonic lethals. If an adult gene exists separately from the embryonic gene, one might expect to recover more alleles affecting only adults. A saturation screen over *en28* to search for such viable *engrailed* alleles might clarify the matter.

The possibility that more than one gene function exists necessitates a careful analysis of the *engrailed* abnormalities to determine which altered function caused them. The following discussion will attempt to deal with both the possibility that *engrailed* abnormalities result from alteration of a single gene function, and that more than one function is involved.

Effects of eliminating *engrailed*

The abnormalities which represent the absence of *engrailed* may prove to be the most informative as to the function of the wild type gene or genes. If the *engrailed* locus is complex, the genotypes must be grouped according to which function is affected. Homozygosis of *en28* causes embryonic lethality (M.A. Russell, personal communication), presumably by eliminating the embryonic function. However, *en28*/+ is normal, indicating that one dose of the embryonic gene is sufficient for normal development. Thus the adult gene function may be studied in trans-heterozygotes of *en28* and alleles which do not affect the embryonic function. Both *en28*/*en30* and *en28*/*en*^{1} fall into this category. Adult abnormalities which are most extreme in these genotypes are the wing to haltere transformation and posterior leg abnormalities (Table 7, Group 2). It is noteworthy that genital abnormalities, independently described by F. Epper (personal communication), also fall into this category.

The common feature of these pattern abnormalities and those originally identified in *en*^{1} homozygotes (Table 7, Group 1) is that the posterior compartments are affected in both cases. Although different subsets of abnormalities are expressed more strongly in different genotypes, the abnormalities are related. This is indi-

cated by the observation that selection of flies with increased expression of some abnormalities can result in simultaneous increase in expression of all others. In addition, a single cell may express more than one type of abnormality, since wing tissue which has been transformed to haltere shows signs of having undergone posterior to anterior transformation as well.

Range of *engrailed* abnormalities

Several inconsistencies are encountered when trying to explain all *engrailed* abnormalities by the same mechanism. Kornberg (1981a,b) has suggested that *engrailed*$^{+}$ is required in the posterior compartment of each segment throughout development to maintain the boundaries between compartments and segments. However, embryonic pattern disruptions are not confined to posterior compartments (Nusslein-Volhard and Wieschaus, 1980; M.A.Russell, personal communication), and there does not appear to be a simple relationship between the embryonic and adult abnormalities. This again makes the hypothesis of separable embryonic and adult gene functions attractive.

It is still not entirely clear whether *engrailed* affects all adult segments. In the head and haltere, any effect is clearly dependent on genetic background. The head effects are unusual in that they have only been found in a single strain of one genotype, and they do not follow the same cold sensitivity pattern of other *engrailed* effects. Possibly their expression requires mutation at another locus, which is abnormal in the single *en*^{1} chromosome showing head effects, and deleted in *en28*. Temperature sensitivity could then be attributed to the second locus.

The transformation of wing tissue to haltere suggests that *engrailed* is in some way involved in regulation of the Bithorax-Complex; improper *engrailed* function results in the activation of the complex in the posterior mesothorax. This transformation is clearly different from that caused by other wing to haltere mutations however. *Contrabithorax* (Lewis, 1963, 1964) and *Polycomb* (Duncan and Lewis, 1982)

produce haltere tissue at the posterior wing margin. The tissue extends anteriorly for varying distances, and can cross into the anterior compartment. Haltere mimic transforms the wing blade to haltere, beginning in the anterior compartment and extending into the posterior. However, engrailed wing to haltere tissue invariably defines the anterior-posterior boundary, and only extends posteriorly. Cbx affects proximal structures (wing hinge and post-notum) whereas engrailed effects are limited to the wing blade. In addition, all other wing to haltere mutations are dominant whereas engrailed is completely recessive. Thus the engrailed wing to haltere transformation does not readily resemble other engrailed abnormalities or other wing to haltere transformations.

Role of the engrailed$^+$ gene

The nature of the abnormalities produced by deletion of engrailed does not support the hypothesis that engrailed is a selector gene. There are at least two broad classes of alternative interpretations of engrailed activity. The first type of explanation assumes that engrailed is involved in some type of regulatory role in adult posterior compartments, and is required for the proper development of these compartments. Disruption of engrailed activity thus disrupts the development of posterior compartments only, but the result of this disruption need not be transformation to anterior compartment. Such an explanation is essentially a less stringent modification of the selector gene hypothesis. It does not, however, clarify the mechanism of engrailed action, nor does it account for the embryonic abnormalities. Such a hypothesis involves assuming that the embryonic phenotype results from a closely linked but functionally unrelated locus. However, abnormality at the embryonic locus must be able to exert an effect on the activation of the adult gene, since clones of en-lethals do cause adult abnormalities. If related gene functions are involved, refinements in regulation must allow expression of the adult gene only

in posterior compartments.

An alternative is that *engrailed* is a trivial gene in a developmental sense, but is required for some basic cellular function. This hypothesis has also been suggested by Kornberg (1981a); it implies that *engrailed* does not have any regulatory role. For example, it may direct production of a component of the cell membrane. Changing the character of the component or eliminating it could alter the permeability of cells, causing molecules to diffuse abnormally. This could result in abnormal pattern differentiation or production of pattern elements in the wrong place if positional information gradients were altered, or even cell death, if essential nutrients were not allowed to diffuse into cells.

The problem with such a general hypothesis is that it does not explain the apparent specificity of adult abnormalities to the posterior compartment. For this, it may be necessary to assume a differential distribution of the component between anterior and posterior compartment. Thus, *engrailed* becomes one of the "cyto-differentiation" genes in the scheme originally proposed by Garcia-Bellido (1975), dependent on another gene to direct its proper distribution. However, such a hypothesis does have the advantage that it can explain both the adult and embryonic abnormalities without postulating multiple gene activities. Changes in the permeability of embryonic cells could easily result in disruptions of gradients required to produce proper segmentation. In addition, different effects would result from eliminating the locus (as in *en28*), from reducing gene activity (as may be occurring in some lethals), or from altering it (as in en^1).

Either a regulatory role, or a general cell function of *engrailed* could be the basis for cell death when the locus was mutant. In some cases, cell death might be followed by duplication of the remaining cells. Evidence of cell death has been presented for the head; it is a factor in the genital abnormalities as well, as shown

by trypan blue staining of the genital disc (Epper and Sanchez, personal communication). Leg abnormalities observed in deficiency genotypes are somewhat reminiscent of those produced by cell death using temperature sensitive cell lethal mutations (Arking, 1975; Simpson and Schneiderman, 1975; Russell et al., 1977; Girton and Russell, 1980). The finding of Lawrence and Struhl (1982) that *en*-lethals are cell lethal in many tissues would also support the hypothesis that cell death plays a role in producing *engrailed* abnormalities.

It is known that compartment commitments are lost, then regained during the process of regeneration following cell death (Schubiger, 1971; Haynie and Bryant, 1976; Tiong et al., 1977; Szabad et al., 1979), which might provide an explanation for the behavior of *engrailed* clones. In addition, cell death in the posterior compartment of the wing disc, followed by regeneration, often results in the production of triple row bristles on the posterior margin (Szabad et al., 1979), thus such apparent posterior to anterior transformations could be explained. This hypothesis alone is not entirely satisfactory, since there is no evidence of extensive cell death in en^1 wing discs. Small en^1/en^1 clones (in $en^1/+$ backgrund) near the posterior wing margin, where they could not have resulted from regeneration by anterior cells, still autonomously differentiate transformed elements such as PTR bristles (Lawrence and Morata, 1976). This indicates that cell death and regeneration is not a prerequisite for the appearence of anterior pattern elements in the posterior compartment. Cell death cannot be invoked to explain the transformation of wing tissue to haltere. The most satisfactory hypothesis for *engrailed* action may be that *engrailed* mutants affect a basic cellular function in a way that sometimes results in cell death.

The effects of *engrailed* are more complex than originally believed. Although *engrailed* clearly does not show all the characteristics of a selector gene, an alternative role for it cannot easily be assigned. Elucidation of the true role of *engrailed* will require further work, including molecular analysis.

BIBLIOGRAPHY

Arking, R.(1975). Temperature sensitive cell lethal mutants of *Drosophila*; Isolation and characterization. Genetics *80*, 519-537.

Auerbach, C.(1936). The development of the legs, wings and halteres in wild type and some mutant strains of *Drosophila melanogaster*. Trans. Royal Soc. Edinburgh, *58*, 787-816.

Balkaschina, E.I.(1929). Ein Fall der Erbhomoosis (die Genovariation "aristopedia") bei *Drosophila melanogaster*. Wilhelm Roux Arch. Ent. Org. *115*, 448-463.

Bateson, W.(1894). "Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of Species". London: MacMillan.

Becker, H.J.(1957). Uber Rontgenmosaikflecken und Defektmutatioen am Auge von *Drosophila* und die Entwicklungsphysiologie des Auges. Z. Indukt. Abstamm. Vererbungsl. *88*, 333-373.

Bodenstein, D.(1950). The postembryonic development of *Drosophila*. In "Biology of *Drosophila*", Ed. Demerec, Wiley and Sons, Inc., New York.

Bownes, M., and J.H. Sang (1974a). Experimental manipulation of early *Drosophila* embryos. I. Adult and embryonic defects resulting from microcautery at nuclear multiplication and blastoderm stages. J. Embryol. Exp. Morphol. *32*, 253-272.

Bownes, M., and J.H. Sang (1974b). Experimental manipulation of early *Drosophila* embryos. II. Adult and embryonic defects resulting from the removal of blastoderm cells by pricking. J. Embryol. Exp. Zool. *32*, 273-285.

Brasted, A.(1941). An analysis of the expression of the mutant *engrailed* in *Drosophila melanogaster*. Genetics *26*, 247-373.

Bridges, C.B., and P.N. Bridges(1939). A new map of the second chromosome. J. Hered. *30*, 475-477.

Brower, D.L., P.A. Lawrence, and M. Wilcox(1981). Clonal analysis of the undifferentiated wing disk of *Drosophila*. Devel. Biol. *86*, 448-455.

Brower, D.L., R.J. Smith, and M. Wilcox(1982). Cell shapes on the surface of the *Drosophila* wing imaginal disc. J. Embryol. Exp. Morph. *67*, 137-151.

Bryant, P.J.(1975). Pattern formation in the imaginal wing disc of *Drosophila melanogaster*: fate map, regeneration and duplication. J. Exp. Zool. *193*, 49-78.

Chan, L.N., and W. Gehring (1971). Determination of blastoderm cells in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. (USA) *68*, 2217-2221.

Crick, F.H.C., and P.A. Lawrence(1975). Compartments and polyclones in insect development. Science *189*, 340-347.

Demerec, M.(1950). "The Biology of *Drosophila*". J. Wiley and Son, Publ., New York.

Duncan, I., and E.B. Lewis(1982). Genetic control of body segment differentiation in *Drosophila*. In: "Developmental Order: Its Origin and Regulation," Alan R. Liss, Inc., New York.

Epper, F.(1980). Annual report of the Developmental Biology Center, University of California, Irvine (1980/81).

Eker, R.(1929). The recessive mutant *engrailed* in *Drosophila melanogaster*. Hereditas *12*, 217-222.

Fausto-Sterling, A., and H. Smith-Schiess(1982). Interactions between *fused* and *engrailed*, two mutations affecting pattern formation in *Drosophila melanogaster*. Genetics *101*, 71-80.

Foe, V.E., and B.M. Alberts(1983). Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. J. Cell Science *61*, 31-70.

Garcia-Bellido, A.(1966). Pattern reconstruction by dissociated imaginal disk cells of *Drosophila melanogaster*. Devel. Biol. *14*, 278-306.

Garcia-Bellido, A.(1975). Genetic control of wing disc development in *Drosophila*. In *Cell Patterning*, CIBA Foundation Symposium *29*, 161-182. Amsterdam: Elsevier.

Garcia-Bellido, A.(1977). Homoeotic and atavic mutations in insects. Amer. Zool. 17, 613-629.

Garcia-Bellido, A., and J. Dapena(1973). Recovery of cell marker mutants in *Drosophila*. DIS 50, 179.

Garcia-Bellido, A., P.A. Lawrence, and G. Morata(1979). Compartments in animal development. Sci. Am. 241, No. 1, 102-110.

Garcia-Bellido, A., P. Ripoll and G. Morata(1973). Developmental compartmentalization of the wing disc of *Drosophila*. Nature New Biology 245, 251-253.

Garcia-Bellido, A., P. Ripoll and G. Morata(1976). Developmental compartmentalization in the dorsal mesothoracic disc of *Drosophila*. Devel. Biol. 48, 132-147.

Garcia-Bellido, A., and P. Santamaria(1972). Developmental analysis of the wing disc in the mutant *engrailed* of *Drosophila melanogaster*. Genetics 72, 87-104.

Gehring, W., and R. Nothiger(1973). The imaginal discs of *Drosophila*. in "Developmental Systems: Insects". Volume 2, Ed. C.H.Waddington, S.Counce, New York: Academic Press.

Grell, E.F.(1969). New mutant report: *Black cells*. DIS 44, 46.

Hadorn, E.(1965). Problems of determination and transdetermination. In "Genetic control of differentiation". Brookhaven Symp. 18, 148-161.

Hadorn, E.(1978). Transdetermination. In: "The Genetics and Biology of *Drosophila*". Vol. 2C. Ed. M. Ashburner and T.R.E. Wright, Academic Press, London, New York, San Francisco.

Hadorn, E., and D. Buck(1962). Ueber Entwicklungsleistungen transplantierter Teilstucke von Flugel-Imaginalscheiben von *Drosophila melanogaster*. Rev. Suisse Zool. 69, 302-310.

Hayes, P.(1982). Mutant analysis of determination in *Drosophila*. Ph.D. Thesis, Department of Genetics, University of Alberta, Edmonton.

Illmensee, K.(1978). Drosophila chimeras and the problem of determination. In "Genetic Mosaics and Cell Differentiation". Ed. W.Gehring, Springer-Verlag, Berlin, Heidleberg, New York.

Illmensee, K., and A. Mahowald(1974). Transplantation of posterior polar plasm in Drosophila. Induction of germ cells at the anterior pole of the egg. Proc. Nat. Acad. Sci. (USA) 71, 1016-1020.

Kauffman, S.A.(1973). Control circuits for determination and transdetermination. Science 181, 310-318.

Kauffman, S.A.(1975). Control circuits for determination and transdetermination: Interpreting positional information in a binary epigenetic code. In: "Cell Patterning". CIBA Foundation Symposium 29, 201-214, Amsterdam: Elsevier.

Kauffman, S.A.(1977). Chemical patterns, compartments, and a binary epigenetic code in Drosophila. Am. Zool. 17, 631-648.

Kornberg, T.(1981a). engrailed: a gene controlling compartment and segment formation in Drosophila. Proc. Nat. Acad. Sci.(U.S.) 78, 1095-1099.

Kornberg, T.(1981b). Compartments in the abdomen of Drosophila and the role of the engrailed locus. Devel. Biol. 86, 363-372.

Lawrence, P.A.(1981a). The cellular basis of segmentation in insects. Cell 26, 3-10.

Lawrence, P.A.(1981b). A general cell marker for clonal analysis of Drosophila development. J. Embryol. Exp. Morph. 64, 321-332.

Lawrence, P.A., and G. Morata(1976). Compartments in the wing of Drosophila: a study of the engrailed gene. Devel. Biol. 50, 321-337.

Lawrence, P.A., and G. Struhl(1982). Further studies of the engrailed phenotype in Drosophila. The EMBO Journal, Vol. 1 No.7, 827-833.

Lawrence, P.A., G. Struhl, and G. Morata(1979). Bristle patterns and compartment boundaries in the tarsi of Drosophila. J. Embryol. Exp. Morph. 51, 195-208.

Lewis, E.B.(1960). A new standard food medium. DIS 34, 117-118.

Lewis, E.B.(1963). Genes and developmental pathways. Am. Zool. 3, 33-56.

Lewis, E.B.(1964). Genetic control and regulation of developmental psthways. In: "The Role of Chromosomes in Development". Symp. Soc. Devel. Biol. 23, 231-252.

Lewis, E.B.(1978). A gene complex controlling segmentation in *Drosophila*. Nature 276, 565-570.

Lindsley, D., and E. Grell(1968). "Genetic Variations of *Drosophila melanogaster*". Carnegie Institute, Washington, Publ. 627.

Lindsley, D.L., L. Sandler, B.S. Baker, A.T.C. Carpenter, R.E.Denell, J.C. Hall, P.A. Jacobs, G.L.G. Miklos, B.K. Davis, R.C. Gethmann, R.W. Hardy, A. Hessler, S.M. Miller, H. Nozawa, D.M. Parry, and M. Gould-Somero(1972). Segmental aneuploidy and the genetic gross structure of the *Drosophila* genome. Genetics 71, 157-184.

Lohs-Schardin, M.(1982). Dicephalic - A *Drosophila* mutant affecting polarity in follicle organization and embryonic patterning. Wilhelm Roux Arch. 191, 28-36.

Lohs-Schardin, M., C. Cremer, and C. Nusslein-Volhard(1979). A fate map for the larval epidermis of *Drosophila melanogaster*: localized cuticle defects following irradiation of the blastoderm with an ultraviolet laser microbeam. Devel. Biol. 73, 239-255.

Lohs-Schardin, M., K.Sander, C.Cremer, T.Cremer, and C.Zorn(1979). Localized ultraviolet microbeam irradiation of early *Drosophila* embryos: fate maps based on location and frequency of adult defects. Devel. Biol. 68, 533-545.

Morata, G., and P. A. Lawrence(1975). Control of compartment development by the *engrailed* gene of *Drosophila*. Nature 255, 614-617.

Morata, G., and P.A. Lawrence(1978). Anterior and posterior compartments in the head of *Drosophila*. Nature 274 473-474.

Morata, G., and P.A. Lawrence(1979). Development of the eye-antenna imaginal disc in

Drosophila. Devel. Biol. 70, 355-371.

Morata, G., and P. Ripoll(1975). Minutes: mutants of Drosophila autonomously affecting cell division rate. Devel. Biol. 42, 211-221.

Muller, H.J.(1932). Further studies on the nature and causes of gene mutations. Proceedings of the Sixth International Congress of Genetics 1, 213-255.

Nash, D., and J. Bell(1968). Larval age and pattern of DNA synthesis in polytene chromosomes. Can. J. Genet. Cytol. 10, 82-90.

Neel, J.V.(1940). The interrelations of temperature, body size, and character expression in Drosophila melanogaster. Genetics 25, 225-250.

Nothiger, R.(1972). The larval development of imaginal disks. In "The Biology of Imaginal Disks". Ed. H. Ursprung and R. Nothiger, Springer-Verlag, New York, Berlin, Heidleberg.

Nothiger, R., A. Dubendorfer, and F. Epper(1977). Gynandromorphs reveal two separate primordia for male and female genitalia in Drosophila melanogaster. Wilhelm Roux Arch. 181, 367-373.

Nusslein-Volhard, C.(1979). Maternal effect mutations that alter the spatial coordinates of the embryo of Drosophila melanogaster. In Determinants of Spatial Organization. Ed. S. Subtelney and I. Konigsberg, Academic Press, New York.

Nusslein-Volhard, C., and E. Wieschaus(1980). Mutations affecting segment number and polarity in Drosophila. Nature 287, 795-801.

Nusslein-Volhard, C., M. Lohs-Schardin, K. Sander, and C. Cremer(1980). A dorsal-ventral shift of embryonic primordia in a new maternal effect mutant of Drosophila. Nature 283, 474-476.

Okada, M., J.A. Kleinman, and H.A. Schneiderman(1974). Chimeric Drosophila adults produced by transplantation of nuclei into specific regions of fertilized eggs. Devel. Biol. 39, 286-294.

Rice, T., and A. Garen(1975). Localized defects of blastoderm formation in maternal effect mutants of Drosophila. Devel. Biol. 43, 277-286.

Russell, M.(1974). Pattern formation in the imaginal discs of a temperature sensitive cell lethal mutant of Drosophila melanogaster. Devel. Biol. 40, 24-39.

Russell, M.(1982). Imaginal discs. In "Handbook of Drosophila development". Ed. Ransom, Elsevier Biomedical Press.

Russell, M., and S. Eberlein(1979). New mutants of engrailed in Drosophila melanogaster. Genetics 91, no. 4, pt. 2, s109.

Russell, M., J.R. Girton, and K. Morgan(1977). Pattern formation in a ts-cell lethal mutant of Drosophila: The range of phenotypes induced by larval heat treatments. Wilhelm Roux Arch. 183, 41-59.

Russell, M., and P. Hayes(1980). The genetics of pattern formation. In Insect Biology in the Future. Ed. M. Locke and D. Smith, Academic Press, New York.

Schubiger, G.(1968). Anlageplan, determinationzustand und transdeterminationleistungen der mannlichen vorderbeinscheibe von Drosphila melanogaster. Wilhelm Roux Arch. Entwicklungsmech. Org. 160, 9-40.

Schubiger, G.(1971). Regeneration, duplication, and transdetermination in fragments of the leg disc of Drosophila melanogaster. Devel. Biol. 26, 277-295.

Schubiger, G., R.C.Moseley, and W.J. Wood(1977). Interaction of different egg parts in determination of various body regions in Drosophila melanogaster. Proc. Nat. Acad. Sci. (USA) 74, 2050-2053.

Simcox, A.A., and J.H. Sang(1983). When does determination occur in Drosophila embryos? Devel. Biol. 97, 212-221.

Simpson, P., and H.A. Schneiderman(1975). Isolation of temperature sensitive mutations blocking clone development in D. melanogaster, and the effects of temperature sensitive cell lethal mutation on pattern formation in imaginal

discs. Wilhelm Roux Arch. 178, 247-275.

Snodgrass(1933). "The Principles of Insect Morphology." McGraw-Hill, New York.

Sonnenblick, B.P.(1950). The early embryology of Drosophila melanogaster. In "Biology of Drosophila", Ed. Demerec, Wiley and Sons, Inc., New York.

Stanley, W.F.(1935). The effect of temperature upon wing size in Drosophila. J. Exp. Zool. 69, 459-496.

Steinberg, M.S.(1964). The problem of adhesive selectivity in cellular interactions. In: "Cellular Mechanisms in Development". Symp. Soc. Study Devel. Growth 22. Ed. Locke. Academic Press, New York, London.

Steiner, E.(1976). Establishment of compartment boundaries in the developing leg disc of Drosophila melanogaster. Wilhelm Roux Arch. 180, 9-30.

Stern, C.(1936). Somatic crossing-over and segregation in Drosophila melanogaster. Genetics 21, 625-730.

Strub, S.(1977). Developmental potentials of the cells of the male fore leg disc of Drosophila. I. Pattern regulation in intact fragments. Wilhelm Roux Arch. Entwicklungsmech. Org. 181, 309-320.

Struhl, G.(1977). Developmental compartments in the proboscis of Drosophila. Nature 270, 723-725.

Struhl, G.(1981). Anterior and posterior compartments in the proboscis of Drosophila. Devel. Biol. 84, 372-385.

Szabad, J., P. Simpson, and R. Nothiger(1979). Regeneration and compartments in Drosophila. J. Embryol. Exp. Morphol. 49, 229-241.

Thierry-Mieg, D.(1982). Paralog, a control mutant in Drosophila melanogaster. Genetics 100, 209-237.

Tiong, S.Y., J.R.Girton, P.H.Hayes, and M.A.Russell(1977). Effect of regeneration on compartment specificity of the bithorax mutant of Drosophila melanogaster. Nature 268, 435-437.

Tokunaga, C.(1961). The differentiation of a secondary sex comb under the influence of the gene engrailed in Drosophila melanogaster. Genetics 46, 157-176.

Turner, F.R., and A.P. Mahowald(1976). Scanning electron microscopy of Drosophila embryogenesis I. The structure of the egg envelopes and the formation of the cellular blastoderm. Devel. Biol. 50, 95-108.

Underwood, E.M., F.R. Turner, and A.P. Mahowald(1980). Analysis of cell movements and fate mapping during early embryogenesis in Drosophila melanogaster. Devel. Biol. 74, 286-301.

Wieschaus, E., and W. Gehring(1976). Clonal analysis of primordial disc cells in the early embryo of Drosophila melanogaster. Devel. Biol. 50, 249-263.

Wieschaus, E., and R. Nothiger(1982). The role of the Transformer genes in the development of genitalia and analia of Drosophila melanogaster. Devel. Biol. 90, 320-334.

Wolpert, L.(1969). Positional information and the spatial pattern of cellular differentiation. J. Theoret. Biol. 25, 1-47.

Wolpert, L.(1971). Positional information and pattern formation. Curr. Topics Devel. Biol. 6, 183-224.

Wolpert, L., and J. Lewis(1975). Towards a theory of development. Fedn. Proc. Am. Soc. Exp. Biol. 34, 14-20.

Zalokar, M.(1971). Transplantation of nuclei in Drosophila melanogaster. Proc. Nat. Acad. Sci. (USA) 68, 1539-1541.

Zalokar, M.(1973). Transplantation of nuclei into the polar plasm of Drosophila eggs. Devel. Biol. 32, 189-193.

Zalokar M, and I. Erk(1976). Division and migration of nuclei during early embryogenesis of Drosophila melanogaster. J. Microscopie Biol. Cell. 25, 97-106.

Zalokar, M., and I. Erk(1976). Division and migration of nuclei during early

Appendix 1. Analysis of variance for expressivity of posterior triple row bristle abnormality.

Source of variation	SS	df	MS	F	p value
Among genotypes	109,830.14	3	30,690.89	$\frac{30690.89}{120.33} = 255.06$	<0.001[1]
Among strains within genotypes	2,045.56	17	120.33	$\frac{120.33}{38.14} = 3.15$	<0.001[2]
Among flies within strains	9,878.91	259	38.14	$\frac{38.14}{20.93} = 1.82$	<0.001[3]
Between wings within flies	5,861.00	280	20.93		

1. p value for 3/17 df = 0.001 when F = 8.73
2. p value for $17/\infty$ df = 0.001 when F = 2.4
3. p value for ∞/∞ df = 0.001 when F = 1.00

Note that although there are significant differences among flies within strains and among strains within genotypes, the major source of variation is the difference among genotypes.

Appendix 2. Analysis of variance for wing shape.

Source of variation	SS	df	MS	F	p value
Among genotypes	1162.44	3	378.48	$\frac{378.48}{30.03} = 12.90$	<0.001[1]
Among strains within genotypes	330.36	11	30.03	$\frac{30.03}{10.54} = 2.85$	<0.005 >0.001
Between wings within flies	1644.88	156	10.54		

[1] p value for 3/11 df = 0.001 when F = 11.56

Note that while there is a significant amount of variation among strains within a genotype, there is greater variation among genotypes.

Appendix 3. Analysis of variance for posterior wing vein abnormality.

Source of variation	SS	df	MS	F	p value
Among genotypes	211.12	3	70.37	$\frac{70.37}{0.34} = 206.36$	<0.001
Among strains within genotypes	6.44	13	0.50	$\frac{0.50}{0.36} = 1.466$	>0.10
Among flies within strains	39.52	109	0.36	$\frac{0.36}{0.34} = 1.052$	>0.10
Between wings within flies	43.00	126	0.34		

Note that there is no significant difference among flies in a strain or among strains in a genotype. All significant differences derive from differences among genotypes.

Appendix 4. Analysis of variance for expressivity of posterior sex comb teeth.

Source of variation	SS	df	MS	F	p value
Among genotypes	3690.86	3	1230.29	$\frac{1230.29}{7.77} = 158.34$	$<0.001^1$
Among strains within genotypes	217.57	28	7.77	$\frac{7.77}{2.98} = 2.61$	$<0.01^2$
Among flies within strains	283.02	95	2.98	$\frac{2.98}{1.84} = 1.62$	$<0.01^3$
Between legs within flies	233.50	127	1.84		

1. p value = 0.001 when $F = 7.19$ for 3/28 df.
2. p value = 0.01 when $F \cong 1.9$ for 28/95 df.
3. p value = 0.01 when $F \cong 1.3$ for 95/128 df.

Note that while there is a significant amount of variation among flies within strains and among strains within genotypes, the major variation derives from the differences among genotypes.

Appendix 5. X^2 test for expected distribution of alula fusion among wings of en^1/en^1 flies.

Total number of flies=30 Total number of wings=60
Number of wings showing fused alula=44 Fusion frequency=0.73

	Expected number of flies	Observed number of flies	X^2
Both wings affected	16.13	19	0.5107
One wing affected	11.72	6	2.7917
Neither wing affected	2.15	5	3.7779
			X^2 = 7.0803

$p < 0.001$ at two degrees freedom.